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THE UNIVERSITY OF ALBERTA

The Effect of Energy Intake on Central and Peripheral Norepinephrine Turnover in Lean Mice

by

© Janis Davis-Street

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

IN

Nutrition

FACULTY OF HOME ECONOMICS

EDMONTON, ALBERTA
SPRING, 1988

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ISBN 0-315-42720-5

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled The Effect of Energy Intake on Central and Peripheral Norepinephrine Turnover in Lean Mice submitted by Janis Davis-Street in partial fulfillment of the requirements for the degree of Master of SCIENCE in Nutrition.

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Date January 19, 1988

Dedication

To My Parents, Harold and Mayleen Davis,
My Husband, Paul,
and
My Brother, Russell.
Thank you for your loving support.

Abstract

The effects of energy intake and food deprivation on central and peripheral norepinephrine (NE) turnover and the role of serum insulin and selected metabolic substrates, namely serum glucose, total free fatty acids (FFA) and tyrosine to other neutral amino acids ratio (tyrosine/other NAA) in mediating the sympathetic response were determined in four-week-old female mice (C57BL/6J, +/+). In order to isolate the effects of energy restriction distinct from those of prolonged deprivation on NE turnover, mice were fed a high carbohydrate (CHO) (58% CHO, 20% fat, 22% protein) diet either ad libitum or restricted to 34 or 24 kJ/d. The restricted intakes were divided into two equal meals daily and fed at the beginning and middle of the dark period to reduce the duration of deprivation accompanying normal energy restriction. NE turnover was determined simultaneously in brain, heart, interscapular brown adipose tissue, pancreas and kidney, from the decline in NE concentration after synthesis inhibition by α methyl tyrosine. NE turnover was similar in both ad libitum and restricted "meal-fed" mice at either level of restriction. When an identical restricted intake of 34 or 24 kJ/d was provided as a single daily meal at the beginning of the dark period, prolonging the duration of deprivation, NE turnover was 38 and 46% lower in the heart alone of these restricted (deprived) mice, compared to ad libitum fed controls. This

suppression of NE turnover with restriction resulting in prolonged deprivation provides a previously unidentified model to test possible mechanisms of diet-induced sympathetic suppression. Serum insulin, glucose, total FFA and tyrosine/other NAA were determined in dietary conditions of sympathetic activation (high carbohydrate and high fat diets) and suppression (high protein diet and prolonged deprivation). Fractional NE turnover rate was not related to any of the serum factors measured. These results suggest that the duration of deprivation predominates in the effect of caloric restriction on sympathetic activity, and not energy content, per se. Moreover, the suppression of NE turnover produced with prolonged deprivation and consumption of a high protein diet is not associated with changes in serum insulin, glucose, total FFA or tyrosine/other NAA.

Acknowledgement

Special thanks to my supervisor, Dr. J.L. Johnston for her endless encouragement in the face of any adversity; to my committee members, Dr. P. Trayhurn and Dr. R. Christopherson for their patient perusal of this thesis; and to my friends and colleagues, especially Barbara Marriage, for their constant support.

This work was supported by the Alberta Heritage Foundation for Medical Research.

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I. INTRODUCTION

The catecholamine neurotransmitter norepinephrine (NE), is involved in many functions, including neuroendocrine function, thermoregulation and blood pressure regulation, via central or peripheral sympathetic nervous system activity (SNS) or both. The role of the SNS in thermogenic function has been well established. Sympathetic mediation of thermogenesis in response to hypothermia and independent of the adaptive mechanisms of muscular activity (shivering thermogenesis), is referred to as non-shivering thermogenesis and has been conclusively demonstrated in the literature (1). More recently, an effect of dietary intake on SNS activity has been identified. Stimulation of SNS activity has been observed with sucrose overfeeding (2-7), suppression of activity accompanying fasting (3-6,8,9). These diet-induced changes have pointed to a possible role for the diet in mediating changes in energy expenditure associated with changes in SNS activity, a phenomenon termed diet-induced thermogenesis (1,10-12).

The similarity in the effect of high fat and high carbohydrate diets (13-16) on SNS activity in rats, when protein content as percentage of energy is held constant, suggests the possibility that caloric content may be a stimulus for peripheral sympathetic activation, as both diets appear to increase NE turnover in sympathetically innervated organs.

The purpose of this review is to examine the evidence for a relationship between energy intake and SNS activity. Using animal and human studies, the role of specific nutrients and caloric content in eliciting SNS activation or suppression will be critically analysed. The putative mechanisms mediating the sympathetic response will be examined, and the functional implications of the diet-induced changes in sympathetic activity will be briefly discussed.

A. Sympathetic nervous system: Definition and assessment

Peripheral organ systems are regulated by the autonomic nervous system(ANS) which innervates the viscera, the endocrine and exocrine glands and all smooth muscle. The ANS consists of two distinct major divisions: the parasympathetic nervous system(PNS) and the SNS. These divisions provide synergistic or opposing effects in their target organs. In general, the PNS maintains homeostasis and conserves the body's resources, while the SNS mediates the body's response to stress.

The functional differences between the SNS and the PNS result in part from anatomic distinctions. The sympathetic ganglia are a collection of neurons arranged in two chains (sympathetic trunks) located close to the spinal cord and receive sympathetic outflow originating in the thoracic and lumbar regions of the spinal cord (Figure I-1). In contrast, the parasympathetic ganglia are generally situated close to

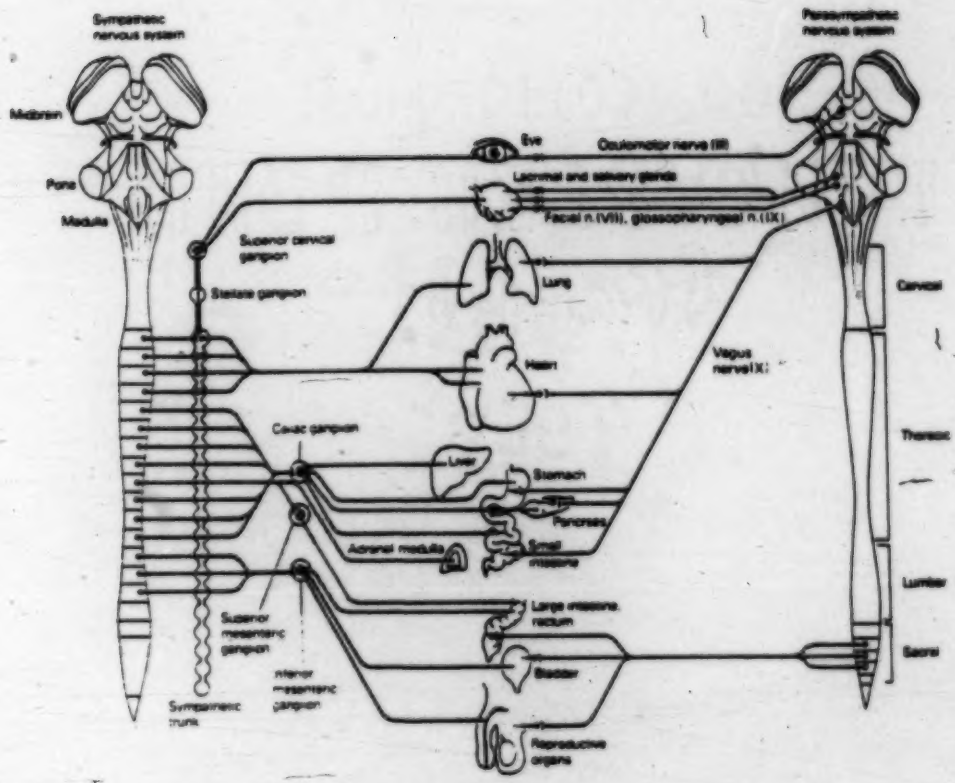

Figure I-1. The autonomic nervous system.

The connections of the PNS and the SNS with the hypothalamus and higher brain centers have been omitted. **Solid lines**, preganglionic axons. **Broken lines**, terminals of postganglionic axons. **Shading** indicates the distribution of the SNS. Sympathetic innervation of blood vessels, sweat glands and piloerector muscles is not shown. Adapted from (17).

the innervated organs, and originate from nuclei in the brain stem and the sacral region of the spinal cord (Figure I-1). The location of the parasympathetic ganglia generally results in the specific innervation of individual organs, compared to the more widespread innervation associated with the SNS. Both divisions of the ANS are regulated by descending fibres of the hypothalamus, which is in turn influenced by the activity of higher brain centers and circulating substrate and hormone concentrations.

The functionally distinct effects of the PNS and the SNS are not only determined by their anatomic segregation, but also by the interaction of their chemical messengers with specific receptors. The response of target organs to changes in sympathetic or parasympathetic activity is determined by changes in receptor function, elicited either by alterations in membrane permeability or by the modification of intracellular metabolism in postsynaptic neurons. Acetylcholine(Ach) is the neurotransmitter of all preganglionic neurons and of the postganglionic neurons of the PNS. Ach reacts with muscarinic and nicotinic receptors, altering intracellular metabolism and membrane permeability, respectively (17). The catecholamine neurotransmitter of the SNS, NE, reacts with α or β adrenergic receptors via changes in intracellular metabolism, through the stimulation of protein kinase C and cyclicAMP synthesis, respectively (17).

NE is synthesized and stored in peripheral sympathetic nerve endings. However, the adrenal medulla provides an

additional source of NE, where it is released along with epinephrine. Quantification of SNS activity should therefore reflect sympathetic sources of NE, distinct from sources of adrenal origin. Evidence for the independent activation of the SNS and adrenal medulla has been furnished by the observation, in rats, of distinct and separate responses to hypoglycemia. Cardiac SNS activity is suppressed by fasting hypoglycemia, but adrenal medullary response is stimulated (9,18-21). Urinary and plasma measurements of NE have proven inadequate, because of their lack of specificity and sensitivity in determining sympathetic activity. Plasma NE measurements are further limited by their dependence on the rate of removal of NE from circulation, as well as on the rate of NE release from sympathetic neurons, and by their inability to detect regional differences in SNS neural activity (22).

Sympathetic activity in animals, is generally determined from the rate of disappearance of radiolabelled NE [^{3}H-NE] from sympathetically innervated organs, or the rate of decline in endogenous NE concentration in these organs after synthesis inhibition with α methyl tyrosine, a competitive inhibitor of tyrosine hydroxylase (23), the rate-limiting enzyme in catecholamine synthesis. The former method has the advantage of allowing equilibration of the radiolabel with endogenous NE without altering the SNS, while metabolic changes may result from the decline in endogenous NE concentration after synthesis inhibition. However, because

the blood-brain barrier is impermeable to [^{3}H-NE], simultaneous measurement of central and peripheral SNS activity is prevented. Although the permeability of the blood-brain barrier to α methyl tyrosine removes this restriction (23), measurement of whole brain turnover is not specific in its reflection of regional differences in central noradrenergic activity; nuclei-specific changes in noradrenergic activity have been observed in mouse brain (24).

The use of α methyl tyrosine or [^{3}H-NE] is based on the assumption that tissue turnover of NE follows steady state kinetics, such that rates of NE synthesis and NE degradation are equal (23,25). The rate of decline of endogenous NE after synthesis inhibition by α methyl tyrosine, is exponential in several tissues (23,26). Further, a single injection of α methyl tyrosine completely inactivates tyrosine hydroxylase for 8 hours and does not appear to alter intra- or extraneural NE metabolism or release (23). The rate of disappearance of [^{3}H-NE] after injection has also been shown to follow first order kinetics because the specific activity of NE exhibits a monoexponential decline over time (25).

The monoexponential decline observed with both methods is in accord with the steady state assumption, and allows estimations of organ specific NE turnover rates (K): from the product of the fractional rate of disappearance (k), derived from the slope of the line of log transformed NE

concentration plotted over time, and the endogenous NE content. Brodie et al (23) have demonstrated almost identical $t_{1/2}$ (half-time of disappearance) values of NE turnover in the hearts of rats using α methyl tyrosine as compared to the radio isotope method. However, the synthesis inhibition technique is limited, as turnover can only be measured for the duration of complete synthesis inhibition (up to 8 hours) (23) compared to 24 hours using [^{3}H-NE].

Compared to the animal data, human studies are limited by their dependence on urinary and plasma measurements of catecholamines and their metabolites as indices of SNS activity. However, newer techniques utilising radiotracer turnover methods have been identified, allowing estimations of NE appearance and clearance rates in plasma (22,27). More recently, these radiotracer techniques have been expanded to allow estimations of total and organ-specific NE kinetics in humans from the determination of [^{3}H-NE] in blood collected from different sample sites (renal vein, dorsal hand vein, hepatic vein, coronary sinus and the antecubital vein) (22).

B. Diet and sympathetic activity

1. Fasting

Studies in rats demonstrate a marked suppression of sympathetic activity with fasting. Cardiac NE turnover, assessed by the rate of disappearance of [^{3}H-NE], is lower than in ad libitum fed controls (by 71, 52, and 43%) (3,6,8) after 2 days of fasting, the standard duration of food

deprivation that has been studied. These data are supported by similar findings using α methyl tyrosine. After 7 hours of synthesis inhibition, endogenous cardiac NE concentration is unaffected by fasting (2,8). Similar results are also observed in the pancreas, liver (3) and brown adipose tissue (BAT) (6) of 48 hour fasted rats (70, 45, and 35% lower NE turnover rates in pancreas, liver and BAT, respectively, than in ad libitum fed controls). These lower NE turnover rates return toward normal values with refeeding (3,8). The lower NE turnover rate with fasting can be detected within the first 24 hours of fasting, as similarly low cardiac NE turnover is observed after 15 or 48 hours of food deprivation (35 and 33% lower than controls) (5). Rappaport et al (9) also report a 34% lower cardiac NE turnover rate in rats restricted to 35% of habitual chow intake for 48 hours. Although these data may point to an independent effect of caloric intake on SNS activity, caloric intake was not systematically manipulated, and a fasting effect accompanying restriction is possible.

The response of the SNS to fasting in humans is not as well defined as in experimental animals. Decreases in plasma free NE and urinary NE metabolites have been observed in obese females after consumption of a semi-starvation diet, providing 9.2 kcal/kg, for 11 (28) and 21 (29) days. As with the changes in the rat studies, these decreases are reversed with refeeding. In contrast to these observations, Leiter et al (30) report a lack of effect of caloric restriction to

400 kcal on plasma NE concentrations. However, unlike the previously reported animal and human studies, where sodium intake was controlled and kept constant during fasting, sodium supplementation was not provided. This confounds the interpretation of these results, as an independent effect of dietary sodium on plasma catecholamine concentration has been previously demonstrated (31). Specifically, diets deficient in sodium (10 meq/d) appear to enhance sympathetic activity in humans (31). The failure of caloric restriction to affect plasma NE levels may therefore reflect a balance between sodium induced increase in plasma NE and the suppressive effects of food deprivation. Although the sensitivity and specificity of the cited indices of human SNS activity are questionable, these studies provide some evidence in support of energy dependent modification of SNS activity in humans.

The suppression of peripheral NE turnover by fasting in animals is undisputed. However, the response of the whole brain to food deprivation is not clear. Rats demonstrate lower NE concentration in the ventromedial and arcuate nuclei, after 48 hours of food deprivation compared to fed controls (32). However, similar deprivation fails to affect NE concentration in these or other hypothalamic nuclei (33). Although measurement of NE concentration may not be reflective of the dynamic state of central adrenergic neurons, turnover studies also provide dissimilar results. NE turnover is increased (34) or unaffected (35) in the

hypothalamus of rats after 22 hours of food deprivation.

Other studies have demonstrated an increase in brain NE concentration, in animals subjected to various types of stress (36,37). Glavin (38) suggests that food deprivation produces stress-induced changes in central adrenergic activity and enhances NE turnover in rat brain. Although stress may be an important factor mediating changes in central NE turnover in response to food deprivation, the literature does not provide convincing evidence supporting this hypothesis. Studies in this laboratory suggest that heterogeneity of brain noradrenergic activity is likely, based on the observation of nuclei-specific responses to food deprivation in mice (24). It appears that, in the brain, some relationship between energy intake and noradrenergic function may exist.

2. Carbohydrate

Stimulation of sympathetic activity by dietary carbohydrate has been demonstrated in experimental animals and humans. Voluntary overfeeding can be induced by offering animals ad libitum access to a sucrose or glucose solution in addition to their habitual laboratory chow intake. However, this feeding paradigm also decreases the proportion of dietary protein, making it difficult to isolate the effects of manipulating dietary carbohydrate. Nevertheless, cardiac NE turnover, assessed by the rate of disappearance of [^{3}H-NE], is greatly enhanced (43, 129, 76, 59% over

controls) by sucrose overfeeding in rats fed 8-10% sucrose solutions in addition to rat chow for 3 days (2-5).

These results are supported by similar findings of sympathetic activation in the hearts of sucrose supplemented rats using α methyl tyrosine (2). Specifically, synthesis inhibition for 7 hours resulted in a significant decrease in endogenous cardiac NE levels with sucrose overfeeding, while fasted and control chow-fed rats were unaffected. This stimulation of the SNS with voluntary sucrose overfeeding was also observed in rat liver and pancreas where NE turnover was 96 and 68% higher, respectively, than in fasted controls (3). NE turnover in BAT was similarly, but non-significantly, higher in sucrose overfed rats compared to chow-fed controls (4).

Evidence for carbohydrate-induced stimulation of SNS activity is also demonstrated by the consumption of simple carbohydrates alone, rather than as supplemental feedings. Cardiovascular stimulation, assessed by an increase in pulse pressure, and a rise in plasma NE concentration, is induced by oral administration of 100g of glucose to normal human subjects (39). Evidence for glucose stimulation of SNS activity in humans is also provided by the rise in oxygen consumption in association with an increase in plasma NE concentration, after ingestion of a similar amount of glucose (40,41). Moreover, this increase in plasma NE appears to be, at least in humans, specific to glucose ingestion, as isocaloric amounts of protein or fat fail to

elicit similar effects on plasma NE concentration (42). However, the limitations associated with the measurement of plasma NE concentration, namely its lack of sensitivity and specificity, as an index of SNS activity, must be considered. More recent observations from the above group demonstrate, in rats, similarly elevated cardiac (197 and 173% above fasted controls) and BAT (120 and 151% above controls) NE turnover, assessed by the rate of disappearance of [^{3}H-NE], after 3 days consumption of single nutrient meals containing either fat or glucose (16).

3. Fat

Consumption of a mixed highly palatable "cafeteria" diet is associated with a significantly higher (by 66% and 108%) cardiac and BAT NE turnover in rats, compared to chow fed controls (6). This dietary paradigm stimulates food intake, particularly of carbohydrate and fat and suggests a possible role for mixed energy macronutrient or fat, stimulation of sympathetic activity. Isocaloric supplementation of a restricted rat chow ration (25% of normal intake) for 5-6 days, with either fat or sucrose providing a more than threefold higher caloric intake, resulted in similarly elevated cardiac NE turnover (148% and 182% for fat and sucrose, respectively) above that in restricted chow-fed controls (13). Although this dietary protocol demonstrates a similar effect of sucrose and fat on NE turnover, it is limited by its inability to allow isolation of the effect of

caloric restriction from overfeeding on sympathetic activity. Moreover, dietary manipulation of carbohydrate and fat clearly affects protein concentration. Fat supplementation of a higher level of restricted chow intake (34% instead of 25%) for 4-5 days, providing a non-significant increase in caloric intake, increased cardiac NE turnover by 50% over ad libitum chow fed controls (13). Because chow intake and hence dietary protein is restricted in the fat supplemented animals, it is again impossible to clearly isolate the effect of fat supplementation from that of lowered protein on SNS activity. Moreover, these results are further confounded because the fat fed animals failed to gain as much weight as their controls.

Modest isocaloric fat or sucrose supplementation of a restricted chow intake (50% of normal intake) for 4-5 days, increasing caloric intake by 50%, resulted in similarly higher NE turnover in heart (52%, 76%), BAT (115%, 42%) but not pancreas, than in restricted chow fed controls (13). Supplementation with isocaloric amounts of fat or sucrose, doubling caloric intake of restricted chow fed controls (37% of normal intake) similarly resulted in higher NE turnover in rat heart (93% and 72%) and BAT (60% and 50%) (13). However, at this level of supplementation NE turnover in pancreas was significantly increased only by the supplemental fat feeding. A significant effect of fat overfeeding on NE turnover is also demonstrated in mice (7). Supplementation of ad libitum chow intake with a 30% sucrose

solution, decreased chow intake by 50% and had no effect on either cardiac or BAT NE turnover, while isocaloric fat supplementation (limited to equal the voluntarily consumed sucrose supplement) of similar chow intake significantly increased turnover in both organs (28% and 34% in heart and BAT, respectively) (7).

This failure of sucrose supplementation to elicit sympathetic activation, conflicts with previously reported observations in rats (13) receiving similar sucrose supplementation. However, sucrose supplementation in this study resulted in a smaller (18% compared to 50% in the rat study) increase in caloric intake. Despite the relative consistency with which sucrose supplementation elicits overfeeding, the 30% sucrose solution may represent an unphysiological challenge, resulting in a form of avoidance behavior. Indeed, supplementation of chow intake with a 30% solution is greatly in excess of the 8-10% sucrose solutions used in previously reported studies (2-5). Although the interpretation of the lack of effect of supplemental sucrose feeding on sympathetic activity in mice is confounded by this observation, the stimulatory effect of fat on NE turnover has been consistently demonstrated (7,13,16). These data suggest that, at certain levels of caloric intake, fat and sucrose elicit organ specific activation of the SNS.

4. Protein

In contrast to fat and sucrose overfeeding, high levels

of protein appear to suppress sympathetic activity in experimental animals. NE turnover in sympathetically innervated organs is decreased by short-term (14,15,25,43) and chronic (14,44,45) increases in dietary protein. Consumption of low protein diets is generally accompanied by replacement of protein by carbohydrate, resulting in high carbohydrate diets (14,15,43,45). This suggests that it may be the relative increase in dietary carbohydrate, a known stimulus for SNS activation, that mediates the sympathetic response.

Rats fed diets deficient in protein ([0%](43), [7-8%] (45), [9.9%](14)) have consistently higher rates of NE turnover in heart (100%, 35-70%, 52% above controls) and BAT (31%, 93-103%, 70%) (14,45), and higher urinary NE excretion (43) compared to control animals fed diets of higher (18%, 22-25%, 39.6%) protein concentration. These increases in sympathetic activity may be due, in part, to stress induced by the consumption of diets with inadequate protein for normal growth (26).

These data are also confounded by the effect of protein concentration on voluntary food intake. Animals on the lower protein diet lost weight (43) or gained less weight (14,44,45) than those receiving higher protein intakes. Moreover, when animals are fed extremes in protein concentration, these dietary manipulations frequently result in varied energy intakes. For example, consumption of protein deficient diets (0%, 5%) decreased energy intake

(31%, 11%) compared to diets containing higher proportions of protein (18%, 15%) (43,44).

In order to isolate the effect of individual energy macronutrients and to control for variations in food intake, Kaufman et al (15) pair-fed rats a fixed amount of chow, supplemented with isocaloric amounts of either sucrose, lard or casein. Cardiac and BAT NE turnover were higher in rats fed diets with supplemental carbohydrate and fat but not protein, suggesting evidence for nutrient-specific stimulation of sympathetic activation. Specifically, supplemental sucrose and lard resulted in higher NE turnover in heart (47-61%) and BAT (61-98%), than in their casein supplemented counterparts, who demonstrated turnover values similar to chow fed controls. Although the stimulatory effects of added sucrose or fat are consistent with earlier reports, the lack of effect of casein supplementation conflicts with previous observations of sympathetic suppression accompanying high protein diets. It is possible that the casein supplemented chow intake represents an unphysiological, predominantly protein, challenge with consequent unpalatability and some degree of avoidance behavior (such that energy intake of the protein supplemented animals was only slightly above chow-fed controls). Energy intake was not rigidly controlled or reported since animals were housed in groups and food intake was adjusted daily to limit chow intake at 5-6g/100g body weight to maintain the pair-feeding paradigm.

Johnston and Balachandran (26) fed mice a high (40%) or adequate (20%) protein diet for 3 days. Total energy intakes and weight gains were similar for both groups of mice. Peripheral NE turnover was higher, by approximately 50% in all organs examined (BAT, heart and kidney) in the low protein fed mice. These data support those previously reported (15,45), and suggest that in circumstances of constant growth and energy intake, decreasing dietary protein concentration, with a concomitant increase in dietary carbohydrate, results in stimulation of sympathetic activity, i.e. a nutrient specific effect independent of energy intake.

5. Energy

The stimulation of sympathetic activity by sucrose overfeeding (2-7) and suppression by fasting (3-6,8,9), provide the clearest evidence for an effect of energy per se on SNS activation in experimental animals. Sucrose and glucose supplementation of habitual chow intake generally promotes an increase in energy intake (2-5). However, these manipulations also change dietary composition and make it difficult to isolate the effects of energy distinct from that of carbohydrate and protein content. In addition, caloric intake in the earlier overfeeding studies was frequently unmonitored as were the effects of sucrose supplementation on chow intake and consequent changes in diet composition (2,3,6).

Cafeteria feeding promoted hyperphagia in rats with concomitant increases in NE turnover in heart and BAT (6). However, these diets are confounded by often unknown diet composition and energy intake.

Fat supplementation also provides a model of overfeeding in experimental animals. The similarity in the effects of isocaloric amounts of fat and carbohydrate, with constant protein (13,16), in stimulating sympathetic activity, provides further evidence in support of energy-mediated SNS activation. However, there are reports of more potent stimulation of the SNS by fat compared to sucrose (7,13).

The effects of protein intake on SNS activity have also been documented. Protein, unlike carbohydrate and fat, appears to suppress peripheral NE turnover. However, this effect has been complicated in some cases by observed variations in energy intake and weight gain that accompany manipulation of dietary protein (14,43,44,45).

The effect of dietary macronutrient content on SNS activity in experimental animals is well documented. However, it remains possible that energy intake is a predominant dietary stimulus for peripheral sympathetic activation. Energy intake is almost always affected by the manipulations of carbohydrate, protein or fat, either by altering palatability and hence voluntary food intake, by the amount of food offered, or by providing unphysiological proportions of the macronutrients, leading to food avoidance or overconsumption.

The effect of overfeeding in normal weight humans has been examined by supplementation of a mixed diet with a combination of carbohydrate, fat and protein (sucrose, cream, gelatin) (46). In subjects overfed by 50% for 20 days without changes in proportions of nutrients, overfeeding failed to affect SNS activity, assessed by the measurement of plasma free NE concentration. This inability of overfeeding to affect plasma NE has been previously observed (47). Moreover, the lack of specificity and sensitivity of plasma NE concentration alone, as an index of SNS kinetics is evident in the above study, as NE appearance and clearance rates rose by 46% and 15%, respectively, in spite of unaltered plasma NE concentration, when normal weight subjects were overfed mixed diets (47). NE appearance and clearance rates were depressed by underfeeding (19% and 14%, respectively), supporting previously reported observations of decreased SNS activity with underfeeding in animals and humans.

The effect of energy intake on sympathetic activity has not been adequately tested. Recent studies have concentrated on the effect of specific energy macronutrients on diets of otherwise equal nutrient density (13-15,25,41-44). Weight changes frequently accompanied these dietary manipulations, confounding interpretation of the results. Systematic dietary manipulation of energy intake from carbohydrate and fat sources, with constant protein concentration, and without changes in body weight, would provide a controlled

test of the effect of energy intake on sympathetic activity.

C. Mechanisms

The involvement of central mechanisms in the regulation of peripheral changes in NE turnover has been demonstrated. Retention of [^{3}H-NE] is increased and decreased, respectively, in situatibns of SNS stimulation (sucrose overfeeding, cafeteria-feeding and cold exposure) and suppression (fasting) after ganglionic blockade with chlorisondamine (3,6). These effects have been demonstrated in the heart, BAT, kidney and pancreas of experimental animals, and provide evidence that observed changes in peripheral NE turnover are reflective of changes in centrally mediated noradrenergic activity.

When normal humans are overfed carbohydrate, alone (48,49), or with other supplements of mixed protein and fat for 20 days, resting metabolic rate increases without a change in plasma and urinary concentrations of NE or NE metabolites. In addition, Seaton et al (50) reported that adrenergic blockade for 3 hours had no effect on metabolic rate at any time following glucose ingestion, compared to saline-treated controls. These results suggest that overfeeding may not influence the sympathetic component of thermogenesis in humans.

Little is known of the central neural mechanisms that coordinate sympathetic function with changes in dietary intake. The involvement of the hypothalamus is suggested by

the presence of connections in the ventromedial hypothalamus(VMH) to brain stem centers, by the importance of several hypothalamic areas in regulation of feeding behavior, and by the role of the hypothalamus in the integration of autonomic and endocrine responses to various plasma constituents (51). Intraperitoneal injection of gold thioglucose, a compound that blocks the uptake of glucose in target cells and purportedly destroys the VMH, abolishes changes in cardiac NE turnover in response to fasting and sucrose overfeeding, situations which respectively suppress and increase SNS activity (4). However, gold thioglucose does not exclusively affect the VMH, and pathologic lesions in extra-hypothalamic sites within the central nervous system have been reported (4). Nevertheless, these data suggest a role for the VMH as a possible central structure that mediates the peripheral response to changes in diet.

Young and Landsberg (51) have suggested a model of dietary regulation of the SNS as follows: central sympathetic outflow is determined by brain stem centers receiving descending inhibitory input from the VMH; fasting increases this inhibition and overfeeding refined sugars or fat has the opposite effect. Although indirect support of this model is provided by the studies involving gold thioglucose treated rats (4), further investigation of the location of the putative "inhibitory" neurons is warranted. Significantly, Marriage (24) observed no changes in hypothalamic NE turnover in any nucleus, except the

paraventricular nucleus(PVN), with fasting. Fasting increased NE turnover in the PVN, while overfeeding failed to affect turnover in any region examined.

The reputed peripheral signals that initiate the central SNS response have not been elucidated. The suppression of sympathetic activity observed with increasing dietary protein concentration in experimental animals, suggests that the availability of tyrosine may be the mechanism mediating sympathetic activation. Increased availability of tyrosine to the brain, with subsequent increases in NE synthesis and turnover, is proposed to induce central stimulation of inhibitory neurons (43). Alternatively, a glucose-insulin model has been proposed for carbohydrate induced sympathetic activation. Glucose metabolism, reflective of the functional state of peripheral carbohydrate metabolism, is proposed to stimulate the SNS.

1. Precursor hypothesis

Tyrosine is the amino acid precursor of NE. Administration of tyrosine to rats has been shown to increase urinary excretion of NE and its metabolites (52). Agharanya and Wurtman (43) suggest that an increase in dietary protein may suppress peripheral NE turnover via an increase in central tyrosine availability and central NE turnover. However, support in the literature for the precursor hypothesis as described, is not forthcoming. Supplementation of low protein diets with tyrosine should

suppress the acceleration in NE turnover associated with low protein feedings. Although the ability of tyrosine supplementation to increase plasma and tissue tyrosine levels is reproducible (26,43,45,53), tyrosine supplementation fails to affect NE turnover in peripheral tissues (26,45,53).

If dietary protein, and hence tyrosine, was the major predictor of sympathetic activation, high protein diets would produce higher plasma and tissue tyrosine levels, enhance brain NE turnover and lower turnover rates in the periphery (26). Johnston and Balachandran (26) assessed plasma tyrosine levels, and tyrosine concentrations and NE turnover in a number of peripheral organs, in mice fed a 40% or 20% protein diet. Protein concentration had no effect on plasma tyrosine concentration in spite of a 50% higher NE turnover in the heart, BAT and kidney of mice fed the 20% protein diet compared to those fed the 40% protein diet. However, energy intake of the mice fed the 20% protein diet transiently exceeded that of those fed 40% protein. In order to assess the contribution of tyrosine availability, intake of the 20% protein fed mice was restricted to 87% of that of the mice fed 40% protein. In spite of the higher plasma and tissue concentrations of tyrosine in the 40% protein fed mice, NE turnover did not differ from the 20% restricted mice in any organs (43).

2. Insulin mediation of sympathetic activity

Landsberg and Young (54) have identified three criteria expected of a physiological signal from the periphery. Firstly, the signal should reflect changes in dietary intake; secondly, experimentally induced changes in the signal, independent of nutrient intake, should produce analogous alterations of SNS activity; and finally, a central mechanism must exist that is capable of recognizing the signal and precipitating the appropriate changes in sympathetic response.

Hypoglycemia is associated with a suppression of SNS activity (5,9,20). However, because plasma glucose levels are maintained within narrow limits, it is unlikely that plasma glucose, per se, is the mediator of sympathetic activity. Intracellular glucose utilization is a more likely candidate for the peripheral signal, as 2-deoxy glucose, a glucose analogue that impairs intracellular glucose metabolism while increasing plasma glucose, appears to suppress the SNS (9).

Insulin occupies a major position in determining glucose metabolism. Evidence for a role for insulin in mediating SNS activation has been provided by studies utilizing glucose and insulin clamp techniques. Stable blood glucose and/or insulin levels can be maintained during continuous and primed infusions of glucose alone, or with insulin, in order to induce conditions of hyperglycemia or hyperinsulinemia (55). These studies reveal a dose-related increase in plasma NE concentration in response to insulin infusions. Moreover,

this effect is greater than that achieved during hyperglycemia and is associated with cardiovascular signs of sympathetic stimulation (55). Similarly, intravenous insulin infusion increases plasma NE concentration in normal humans during normoglycemia, maintained by euglycemic clamping, without effect on adrenal catecholamine release (56).

Minaker et al (57) assessed the effect of hyperinsulinemia on SNS activity, independent of changes in blood glucose, in a group of elderly subjects compared to younger controls by the insulin clamp technique. Insulin infusions failed to increase plasma NE concentration in the older subjects, but elicited slight increases in cardiovascular stimulation and pulse pressure. Conversely, plasma NE concentration and pulse pressure was increased in the younger controls. Although limitations are associated with this index of sympathetic activity, it is possible that the lack of response to insulin in older subjects may be reflective of decreased noradrenergic responsiveness to insulin with age.

In contrast to intravenously administered insulin, oral glucose ingestion produces higher increases in plasma NE concentration in older subjects compared to their younger controls (58). This effect of administration technique suggests that, in the elderly, splanchnic factors (such as vascular, osmotic, neural or hormonal effects in the splanchnic bed), predominate over insulin in mediating SNS stimulation (57). This is not surprising, as insulin

sensitivity, possibly via an effect on receptor number and/or kinetics, is known to decrease with age (58,59).

A requirement for insulin in diet-induced thermogenesis has also been implied by the failure of cafeteria feeding to induce changes in oxygen consumption or responses to NE in streptozotocin-diabetic rats (60). Diet-induced thermogenesis was not stimulated by replacement doses of insulin at 2 levels (2 and 4 units every other day). However, acute doses (8 units) resulted in resting and catecholamine stimulated thermogenesis.

Euglycemic and hyperglycemic insulin clamping in healthy young human subjects produces a dose-related increase in energy expenditure in response to glucose infusion (61). Insulin infusions were significantly correlated with increases in plasma NE concentration, with a slight increase in energy expenditure, findings consistent with previous observations. However, because pharmacological insulin levels are required to elicit significant effects on energy expenditure during euglycemia, it appears that, in physiological conditions, the thermic effect of insulin, mediated by the SNS, is negligible (61). These observations suggest that questions remain unanswered as to the true role of insulin in diet-induced stimulation of the SNS.

Similar sympathetic activation by high fat and high carbohydrate feeding suggests an alternate peripheral signal for sympathetic activation accompanying high fat feeding. Welle and Feldman (16) observed similar increases in NE

turnover in rats fed glucose or fat, with serum insulin and glucose being higher in glucose fed rats only. Ingestion of fat or acute elevation of plasma fatty acids does not stimulate a significant insulin response in man (62). Moreover, high fat diets appear to delay the increases in plasma concentrations of glucose and insulin seen in humans fed a standard diet containing moderate levels of both carbohydrate and fat (63). Conversely, high carbohydrate feeding produces marked elevations in plasma glucose and insulin, similar to those produced by the standard diet. High fat feeding does not appear to stimulate sympathetic activity through any direct effect on insulin or glucose, but may elicit its effect by the release of some other hormone or metabolite.

D. Implications

The existence of diet-induced thermogenesis or its importance as a contributor to energy balance in humans remains controversial (46,48,49). However, evidence supporting a role for diet-induced changes in the SNS in regulating alterations in oxygen consumption and heat production has been provided by animal experiments. Changes in BAT NE turnover that occur with overfeeding or fasting have pointed to a role for BAT as a tissue that mediates the thermogenic response in these animals (6). In addition, the role of other tissues cannot be excluded, as changes in cardiac NE turnover may mediate parallel changes in oxygen

consumption and metabolic rate, and generalised sympathetic stimulation (or suppression) is frequently produced in response to dietary changes. Moreover, thermogenic responses to changes in dietary intake are also dependent on other thermogenic mechanisms such as those induced by the thyroid hormones and Na^+K^+ATPase activity.

Caloric restriction results in suppression of the SNS which may produce consequent decreases in thermogenic function. Moreover, manipulation of macronutrient composition demonstrates that overfeeding fat or carbohydrate stimulates the SNS, which may result in dissipation of excess calories. These diet induced changes may therefore have significant implications for weight control.

The influence of dietary intake on the SNS raises the possibility that physiological and pathophysiological changes associated with diet, may in part be mediated by changes in NE turnover. The implications of diet-induced changes in SNS to obesity and hypertension have been reviewed elsewhere (64). However, the significance of sympathetic changes resulting from changes in energy intake will be discussed.

If caloric content is the major stimulus for SNS activation, caloric restriction in the treatment of obesity may result in a depression of sympathetic activity, limiting the effectiveness of weight-reducing diets due to the possible decreases in thermogenic stimulation. This may have

relevance in the treatment of a sub-group of the obese who are resistant to weight loss, even when food intake is reduced. On the other hand, the increase in sympathetic activity that accompanies refeeding may explain the occurrence of cardiovascular complications observed in individuals during this period. The beneficial effects of weight reduction have been suggested for the management of overweight hypertensive patients. Caloric restriction in humans produces decreases in blood pressure, which may be mediated in part by a decrease in sympathetic activity.

The physiological significance of the dietary influences on sympathetic activity tested to date is limitless. Because of the multiplicity of roles of the SNS in energy expenditure, blood pressure regulation and endocrine function, diet mediated changes may have important implications in both the development and treatment of obesity and hypertension.

E. Summary and conclusions

The effect of dietary intake on SNS activity has been indicated by alterations in NE turnover in a number of sympathetically innervated organs. It has recently been demonstrated that sucrose overfeeding and fasting stimulate and suppress, respectively, NE turnover in rats. Evidence that energy intake affects sympathetic activity is further indicated by the similarity in the effect on NE turnover of high fat and high carbohydrate feedings in rats, when

protein content as percentage of energy is held constant. Both diets increase NE turnover in sympathetically innervated organs. Additionally, NE turnover is increased when a diet low in protein is fed, while high protein diets suppress NE turnover in rats. However, this effect is complicated by the increased carbohydrate content and variation in energy intake that occur with low protein feedings.

In humans, caloric restriction is associated with a reduction in plasma concentration and urinary excretion of NE and its metabolites, when sodium intake is adequate and controlled. Glucose administration increases plasma NE concentration and is accompanied by cardiovascular stimulation and increased oxygen consumption. However, the lack of specificity and sensitivity of plasma and urinary levels, limits their use as indices of sympathetic activity in humans.

The results of the studies reviewed suggest that increasing energy is a predominant dietary stimulus for peripheral sympathetic activation. Insulin, through its effect on glucose utilization has been suggested as the route through which glucose, and hence carbohydrate, has its effect on SNS activity. However, the role of dietary fat in this mechanism is unclear since fat does not appear to affect insulin release. Alternatively, dietary protein content, and through it, tyrosine availability, has been proposed as the mechanism mediating the sympathetic

response. However, this hypothesis has not been supported in the literature.

The effect of energy intake on sympathetic activity has not been adequately tested. Recent studies have concentrated on the effects of specific nutrients, and have been accompanied by changes in body weight and alterations in voluntary food intake. The importance of the SNS in regulating a myriad of physiological processes, suggests that diet-induced changes may have important implications in a number of pathophysiological conditions.

F. Objectives of the present study

The objectives of this study were first, to investigate the relationship between energy intake and deprivation and central and peripheral NE turnover in normal mice; and second, to examine the correlations between serum insulin, glucose, free fatty acids and tyrosine availability to NE turnover under circumstances of sympathetic activation and suppression. It is hypothesized that energy intake provides a distinct stimulus for diet-induced changes in sympathetic activity. Therefore, it is hypothesized that restriction of energy intake in the present study will result in a generalised reduction in peripheral NE turnover. Second, it is hypothesized that serum insulin is associated with NE turnover under circumstances of diet-induced sympathetic activation and suppression.

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II. THE EFFECT OF ENERGY INTAKE ON CENTRAL AND PERIPHERAL NOREPINEPHRINE TURNOVER IN LEAN MICE

A. INTRODUCTION

The catecholamine neurotransmitter norepinephrine (NE) is involved in many functions, including neuroendocrine function, thermoregulation and blood pressure regulation, via central or peripheral sympathetic nervous system activity (SNS) or both. An effect of dietary intake on SNS activity has been indicated by diet-induced changes in NE turnover in a number of sympathetically innervated organs with possible functional effects on thermoregulation. Animal studies demonstrate a possible role for these diet-induced changes in mediating changes in energy expenditure, a phenomenon termed diet-induced thermogenesis (DIT) (1). Because of the possible implications of DIT as a regulator of energy balance, it becomes important to identify what dietary stimuli initiate the sympathetic response.

It has recently been demonstrated that sucrose overfeeding (2-4) and fasting (3-5) stimulate and suppress, respectively, NE turnover in rats. These observations provide the clearest evidence in support of an effect of energy intake on sympathetic activity in experimental animals. Evidence that energy intake affects sympathetic activity is further indicated by the similarity in the effect on NE turnover of isocaloric high fat and high

carbohydrate feedings in rats, when protein content as percentage of energy is held constant (6-9). Both diets increase NE turnover in sympathetically innervated organs, when compared to chow fed controls. However, these manipulations affect the intake of all nutrients, and make it difficult to attribute the effects to any particular nutrient, or energy intake per se.

Additionally, NE turnover is higher in rats fed diets low in protein (0-9.9%), than those fed high protein diets (18-39.6%) (7,10-13). However, attributing this effect to either a specific energy macronutrient or to energy intake itself is difficult because of the concomitant changes in carbohydrate content (7,10,13) and also by the variations in total intake (10,12,13) and body weight (7,10,12,13) that occur with the manipulation of dietary protein. Moreover, energy intake is almost always affected by the manipulation of the macronutrients, either by altering palatability and hence voluntary intake, or by the restrictive effects of controlling energy intake as part of the dietary paradigm. However, in one study when protein concentration was fed within a physiological range (20% versus 40% by weight protein) resulting in constant energy intake and body weight gain, dietary protein per se was shown to affect NE turnover in heart, kidney and interscapular brown adipose tissue (IBAT) of mice (11). When energy intake of the 20% protein diet was subsequently restricted to 87% of that of the 40% protein diet, NE turnover in the same tissues was reduced to

equal that of the 40% protein fed mice (11). Although these data suggest an interactive effect of energy and protein intake on sympathetic activity, the effect of energy intake per se on sympathetic activity, unconfounded by variations in macronutrient composition, or by the effects of deprivation when energy intake is controlled, has not been tested.

The results to date suggest that energy intake, in addition to protein concentration, may be major stimuli for peripheral sympathetic activation by mechanisms unknown. Insulin, through its effect on glucose metabolism, has been suggested as a possible mechanism of carbohydrate-induced sympathetic activation (14,15). Because insulin levels are generally reflective of the assimilation of the total caloric load, especially that of carbohydrate, Landsberg and Young (14) suggest that insulin may act as a central signal of nutrient assimilation, much like its role in the periphery. However, the role of insulin in dietary fat induced sympathetic activation is unclear, since fat does not appear to elicit a significant insulin response (16). It is possible that high fat feeding may stimulate sympathetic activity through the release of some other hormone or metabolite.

Alternatively, the suppression of sympathetic activity with increasing dietary protein concentration observed in experimental animals, has led to the suggestion that the availability of tyrosine in plasma and brain may be the

mechanism mediating sympathetic activation. Specifically, an increase in dietary protein concentration and hence dietary tyrosine intake, has been proposed to lead to the increased availability of tyrosine in the blood, relative to the other large neutral amino acids with which tyrosine competes for access across the blood-brain barrier (10,17). The subsequent increase in brain tyrosine availability then produces an increase in central NE synthesis and turnover, leading to the central stimulation of inhibitory neurons, and the subsequent inhibition of peripheral sympathetic outflow (10). However, recent evidence refutes this proposed mechanism, as tyrosine supplementation of either a high or a low protein diet failed to affect NE turnover in brain, heart, kidney or IBAT, in spite of elevations in plasma and tissue tyrosine concentrations (11). In addition, the decrease in NE turnover in peripheral tissues observed when a high protein diet is fed, is not accompanied by elevations in either brain tyrosine concentration or brain NE turnover (11).

Thus the first objective of this study was to investigate the effects of energy intake and food deprivation on central and peripheral NE turnover. The second objective was to examine the role of insulin and certain other metabolic substrates, namely serum glucose, total free fatty acids and tyrosine relative to the remaining neutral amino acids, in mediating any sympathetic response. Specifically, the effect of energy intake from

mainly carbohydrate or fat sources at two levels of energy intake on central and peripheral NE turnover was investigated. To isolate the effect of energy restriction distinct from that of food deprivation, NE turnover was compared between mice fed the high carbohydrate diet, ad libitum, and restricted mice fed the same diet as two meals daily, or one meal daily. Additionally, the relationships between serum concentrations of insulin, the nutrient substrates glucose, free fatty acids and neutral amino acids and fractional NE turnover rate, were investigated in dietary conditions known to affect NE turnover.

NE turnover in all experiments was determined in brain, IBAT, heart, kidney and pancreas. These organs were selected because of their involvement in thermoregulation, endocrine function and blood pressure regulation, three highly integrated processes controlled by the SNS. Stimulation of the SNS in these organs modifies the endocrine environment to support direct cellular and tissue effects through the synthesis and release of NE (18). In brain, NE is implicated in food intake control and in the central regulation of brown adipose tissue thermogenesis. NE-stimulated thermogenesis in IBAT is mediated by β adrenergic receptors, via the subsequent activation of a unique mitochondrial proton conductance pathway, resulting in an uncoupling of oxidative phosphorylation with resultant heat production. In heart, NE stimulates the cardiovasculature, via α and β adrenoceptors, to redistribute cardiac output, ensuring the

delivery of mobilised substrates to metabolizing tissues. In kidney, NE enhances sodium reabsorption directly and indirectly by the stimulation of the renin-angiotensin and aldosterone system (18). Finally, NE inhibits and stimulates pancreatic insulin secretion via α adrenergic and β adrenergic mechanisms, respectively.

B. Methods

1. Animals and diets

Four week old lean female mice (C57BL/6J+/+, Jackson Laboratories, Bar Harbor, ME) were used in all experiments. They were housed in individual hanging wire-mesh cages in a temperature controlled room (23 $\pm$ 2^{o}C), artificially lit for 12 hours daily. The light/dark cycle commenced at 0900 in experiments 1 to 3, at 0800 in experiment 4, and at 2000 in experiment 5. (The different light regimens were chosen for logistical purposes.) The mice were allowed ad libitum access to water throughout all experiments. One week prior to each experiment, they were adapted to a purified control diet containing 22% of metabolizable energy as protein, 58% as carbohydrate and 20% as fat - the high carbohydrate diet (Table II-1), and then were randomly assigned to the experimental diets. Ad libitum intakes of the control diet and a high fat diet (22% protein, 20% carbohydrate, 58% fat) (Table II-1) were determined from a preliminary experiment (Table II-2). Protein concentration was the same in both diets to control for the effect of variable protein

Table II-1.

COMPOSITION OF THE EXPERIMENTAL DIETS[1]

	High Carbohydrate		High Fat	
	g/100g	%kcal	g/100g	%kcal
Casein[2]	21.1	22	27.8	22
Dextrose	60.6	58	26.6	20
Corn oil	8.2	20	32.3	58
		g/kcal		g/kcal
AIN mineral mix[3]	3.7	0.010	4.9	0.010
AIN vitamin mix[3]	1.9	0.005	2.5	0.005
Cellulose	4.2	0.011	5.6	0.011
Choline bitartrate	0.3	0.001	0.3	0.001

[1] The energy densities of the diets were 3.79, 4.99 and 4.15 kcal/g for the high carbohydrate, the high fat and the high protein (11) diets, respectively, based on 4, 3.64, 4, and 9 kcal/g metabolizable energy for casein, dextrose, starch (11) and corn oil, respectively.

[2] Casein is 87% protein.

[3] Recommendations of Report of the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies (19).

concentration on sympathetic activity (7,8,10,13).

In experiment 1, mice were fed a single meal daily at the beginning of the dark period, containing either 52kJ/day (energy intake equivalent to the observed ad libitum intake of the high fat diet in the preliminary experiment) or a 35% restriction (34kJ/day) of the high carbohydrate or the high fat diet. This level of restriction approximates the level shown to decrease body fat content and increase longevity in normal rats (20). In experiments 2 and 3, mice were fed the high carbohydrate diet ad libitum or restricted to 34kJ/day fed as two daily meals (experiment 2) or as a single daily meal (experiment 3). In experiment 4, mice were fed the high carbohydrate diet either ad libitum, or restricted to 24kJ fed either as a single daily or as two daily meals. In experiment 5, mice were fed one of six diets: the high carbohydrate diet as in experiment 4, the high fat diet ad libitum or restricted to 24kJ fed as a single daily meal, or a high protein diet (44% protein, 34% carbohydrate, 22% fat) (11) ad libitum. Mice were fed the experimental diets for three days in experiments 1 through 5. In all experiments, food intake, corrected for spillage, was monitored every second day during the adaptation period, and daily when the experimental diets were fed. Body weights were measured on the first day of adaptation and on the first and third days of the experimental period.

2. Experimental protocol

Experiment 1

Mice (n=18/treatment) in each of the four treatment groups, those fed the high fat-52kJ, the high fat-34kJ, the high carbohydrate-52kJ or the high carbohydrate-34kJ diets, received their daily intake as a single meal presented at the beginning of the dark period. Sympathetic activity was determined from NE turnover calculated from the rate of decline in NE concentration over 6 hours after synthesis inhibition with α methyl tyrosine (21). On day 4 at 0900, mice were injected i.p. with α methyl tyrosine (α methyl D, L-tyrosine methyl ester hydrochloride, Sigma Chemical Co., St. Louis, MO), (400 mg/kg, in 0.9% saline) and killed by decapitation 0, 3, 6 hours after injection.

Brain (sectioned between right and left hemispheres), IBAT, heart, pancreas and kidney (capsule removed) were rapidly dissected, blotted with tissue to remove excess blood, weighed, wrapped in aluminum foil, frozen on dry ice and held at -40°C until analysis for NE content within 1 month.

Experiment 2

Because all mice in experiment 1 lost weight (Table II-4), it was concluded that controlling "ad libitum" intakes resulted in energy deprivation. To isolate the effect of energy intake from that of deprivation, the mice (n=18/treatment) were fed the high carbohydrate diet ad libitum, or the restricted intake (34kJ) divided into two

meals daily, one at the beginning and one at the middle of the dark period, to reduce the duration of deprivation occurring with normal energy restriction. NE turnover was determined as in experiment 1.

Experiment 3

Mice (n=18/treatment) received the high carbohydrate diet ad libitum or the restricted 34kJ/day, fed as a single meal at the beginning of the dark period, to determine the effect of prolonged deprivation on NE turnover. NE turnover was determined as described in experiment 1.

Experiment 4

Because the ad libitum intakes in experiments 2 and 3 varied, the degree of restriction also varied. It was therefore difficult to separate the effects of duration of deprivation and caloric restriction from those of the relative differences in ad libitum intakes. In order to clarify the sympathetic response to restriction, mice (n=18/treatment) were fed the high carbohydrate diet ad libitum, the high carbohydrate diet restricted to 24kJ daily as a single meal presented at the beginning of the dark period (high carbohydrate restricted group), or the high carbohydrate diet restricted to 24kJ daily fed as two identical meals presented at the beginning and middle of the dark period (high carbohydrate "meal-fed" restricted group). This further level of restriction would provide a more

stringent test of the effects of fasting and caloric restriction on sympathetic activity. NE turnover was determined as in experiment 1 beginning at 0800.

Experiment 5

Mice (n=18/treatment) received one of six dietary treatments:

1. The high carbohydrate diet fed ad libitum.
2. The high carbohydrate diet restricted and meal fed as two meals daily, as in experiment 4.
3. The high carbohydrate diet restricted and fed as one meal daily, as in experiment 4.
4. The high fat diet fed ad libitum.
5. The high fat diet restricted to a daily caloric intake equal that of the high carbohydrate restricted groups (24kJ) fed as a single meal at the beginning of the dark period.
6. The high protein diet fed ad libitum.

On day 4, beginning at 2000, mice were killed within one hour by decapitation and blood was drained from the thoracic stump into untreated test tubes held on ice. Blood from 3-4 mice within each treatment was pooled. Samples were allowed to clot, after which they were centrifuged for 20 min. at 15^{o}C to separate the serum. The serum was rapidly removed, and divided into aliquots held at -40^{o}C for subsequent determination of insulin, glucose, total free fatty acid and

large neutral amino acid concentrations, within 2 months.

3. Analytical methods

NE: NE in all organs was isolated from perchloric acid supernatants of tissue homogenates by alumina extraction under alkaline conditions as previously reported (22). NE was then eluted from the alumina by the addition of perchloric acid (Appendix 1). Detection of NE was achieved by reverse phase high performance liquid chromatography (model 2000, Varian Canada Inc., Georgetown, Ont.) with electrochemical detection (model LC4, Bioanalytical Systems Inc., West Lafayette, IN), using a mobile phase of 0.075M monochloroacetic acid with 250mg/L sodium octyl sulphate as an ion-pairing agent. The mobile phase was delivered at room temperature at 1.7 mL/min.

Glucose: Serum glucose was determined in duplicate by a glucose analyzer based on the glucose oxidase method (Beckman Instruments, Inc., Fullerton, CA) (23) (Appendix 2).

Total Free Fatty Acids: Serum fatty acids were determined by a standard enzymatic colorimetric method (NEFA C Wako Chemicals USA, Inc., Dallas, TX). The test principle of this method is based on the formation of a red quinone dye produced by the oxidative condensation of peroxide, formed by the oxidation of acyl coA synthesised in the presence of

ATP and free fatty acids (24) (Appendix 3).

Neutral amino acids: Serum concentrations of tyrosine, valine, isoleucine, leucine, tryptophan and phenylalanine were determined by high performance liquid chromatography (model 5000, Varian Canada Inc., Georgetown, Ont.) with fluorometric detection of o-phthaldialdehyde derivatives of these amino acids (Fluorichrom model 430020) (25,26) (Appendix 4).

Insulin: Serum insulin was determined by a standard double antibody radioimmunoassay method by a kit (Pharmacia Diagnostics, Uppsala, Sweden) (27) (Appendix 5).

4. Statistical Analyses

NE data were plotted semi-logarithmically with NE turnover being calculated by linear regression of the logarithm of NE concentration over 3 time points (0,3,6 hours). The slopes (b) of the regression lines were calculated by the least squares method. NE turnover rate (K) was calculated as the product of the fractional turnover rate (k) (k=b/0.434) and the estimated endogenous NE concentration at time 0 [NE_o] of each organ (21). Fractional turnover (k x 100%) was expressed as %h: standard error (SE) of k (SE_k) equalled the SE for b (SE_b) divided by 0.434. Comparison of the slopes of the regression lines was made using the variance estimated for the difference between

slopes of the regression equations (28).

The effect of diet on endogenous NE was compared by two-way analysis of variance to determine the significance of the main effects and the interaction of diet composition and energy intake (experiment 1), by one-way analysis of variance followed by Duncan's Multiple Range Tests (experiments 1, 4 and 5) and by students t-test for unpaired variables in two-tailed tests of the null hypothesis (experiments 2 and 3) (29). In experiment 5, the effects of diet on serum glucose, neutral amino acids, free fatty acids and insulin concentrations were compared by one-way analysis of variance followed by Duncan's Multiple Range Tests (28). Simple correlation coefficients were determined in a post hoc correlation of serum parameters with fractional NE turnover rates. Simple and partial correlation coefficients were also determined for the correlation of energy intake, body weight and fractional NE turnover rate. All values are expressed as means $\pm$ SEM.

C. Results

Preliminary Experiment. The observed ad libitum intakes of the high carbohydrate and high fat diets are presented in Table II-2. Although the high carbohydrate fed mice ate 22% more food than their high fat fed counterparts, the higher energy density of the high fat diet resulted in an 8% greater energy intake in mice fed the high fat diet.

Preliminary Experiment
Table II-2.

Observed ad libitum intake of high carbohydrate and high fat diets, and calculated 35% restriction of ad libitum intake

	High Carbohydrate		High Fat	
	(g/d)	(kJ/d)	(g/d)	(kJ/d)
Ad libitum intake[1]	3.03±0.13[2]	48.23	2.48±0.07*	51.98
35% restriction	1.97	31.36	1.61	33.74

[1] Based on food intake monitored for 3 days after 1 week of adaptation to the high carbohydrate diet.

[2] Values are means ± SEM; n=6.

* Significantly different from intake of high carbohydrate diet ($p < 0.001$).

Experiment 1. Mice were fed the high fat or the high carbohydrate diet at either the previously determined ad libitum intake of the high fat diet (52kJ) or a 35% restriction (34kJ). Mice fed the restricted intakes (34kJ) of either the high carbohydrate or the high fat diet had, respectively, 9% and 7% lower heart weights than their corresponding 52kJ fed counterparts (Table II-3). The effect of energy restriction on pancreatic weights was likewise significant. Kidney weights were affected by both diet and energy restriction. Mice fed the high fat diet had heavier kidneys than those fed the high carbohydrate diet, and the mice fed 52kJ of either diet had heavier kidneys than those fed 34kJ, but there was no interaction of diet composition and energy intake. Neither diet nor restriction had an effect on either brain or IBAT organ weights (Table II-3).

Fractional turnover in all organs studied was unaffected by either energy intake or diet composition (Figures II:1-4, Table II-3). Endogenous NE concentration was likewise unaffected, except in IBAT, where NE concentration in mice fed the high carbohydrate diet was almost two times higher than in mice fed the high fat diet (Table II-3).

Controlling the food intake resulted in weight loss in all groups irrespective of diet composition (Table II-4), indicating that all animals were deprived (i.e mice fed 52kJ of either diet received energy intakes inadequate for weight gain). Weight loss in both groups of mice fed 34kJ was more

than 4 times that of those fed 52kJ of either the high carbohydrate or the high fat diet (Table II-4).

Experiment 1
Table II-3.

Norepinephrine(NE) turnover at two levels of energy intake of high carbohydrate or high fat diets

Group	Weight (mg)	NE (nmol/g)	Fractional NE (k) turnover (%h)	Calculated turnover rate (nmol/g/h)
		BRAIN		
High CHO (52kJ)	202.9±3.9[1]	3.39±0.15	11.1±0.01	0.361
High CHO (34kJ)	198.5±3.4	3.50±0.16	12.9±0.01	0.442
High Fat (52kJ)	196.4±3.9	3.40±0.13	12.2±0.01	0.397
High Fat (34kJ)	200.4±5.4	3.53±0.18	14.5±0.01	0.509
		HEART		
High CHO (52kJ)	88.6±1.4^{a}	4.82±0.22	18.5±0.01	0.877
High CHO (34kJ)	80.5±1.4^{b}	4.97±0.22	16.7±0.01	0.840
High Fat (52kJ)	88.3±1.6^{a}	4.61±0.26	16.8±0.01	0.745
High Fat (34kJ)	82.3±1.7^{b}	4.83±0.30	20.9±0.01	1.030
		KIDNEY		
High CHO (52kJ)	99.0±2.5^{bc}	4.71±0.19	21.3±0.02	1.001
High CHO (34kJ)	93.3±2.3^{c}	5.28±0.37	15.8±0.01	0.804
High Fat (52kJ)	109.0±2.2^{a}	4.82±0.37	25.0±0.02	1.248
High Fat (34kJ)	102.6±2.4^{ab}	4.66±0.21	19.7±0.01	0.928
		PANCREAS		
High CHO (52kJ)	107.1±3.1^{ab}	2.64±0.11	18.9±0.02	0.454
High CHO (34kJ)	98.9±2.8^{b}	2.98±0.17	22.8±0.02	0.645
High Fat (52kJ)	114.3±5.2^{a}	2.64±0.40	18.0±0.01	0.408
High Fat (34kJ)	104.6±3.0^{ab}	2.70±0.11	21.3±0.01	0.539
		IBAT		
High CHO (52kJ)	142.7±5.5	2.60±0.28^{a}	15.1±0.03	0.310
High CHO (34kJ)	127.5±5.3	2.76±0.29^{a}	21.3±0.02	0.215
High Fat (52kJ)	146.0±8.1	1.35±0.14^{b}	14.3±0.02	0.174
High Fat (34kJ)	134.0±6.8	1.67±0.23^{b}	22.8±0.02	0.337

[1] Values are means ± SEM; n=18; values in columns with a different superscript are significantly different ($p < 0.05$).

Experiment 1
Table II-4.

Effect of two levels of energy intake of high fat or high carbohydrate diets on weight change

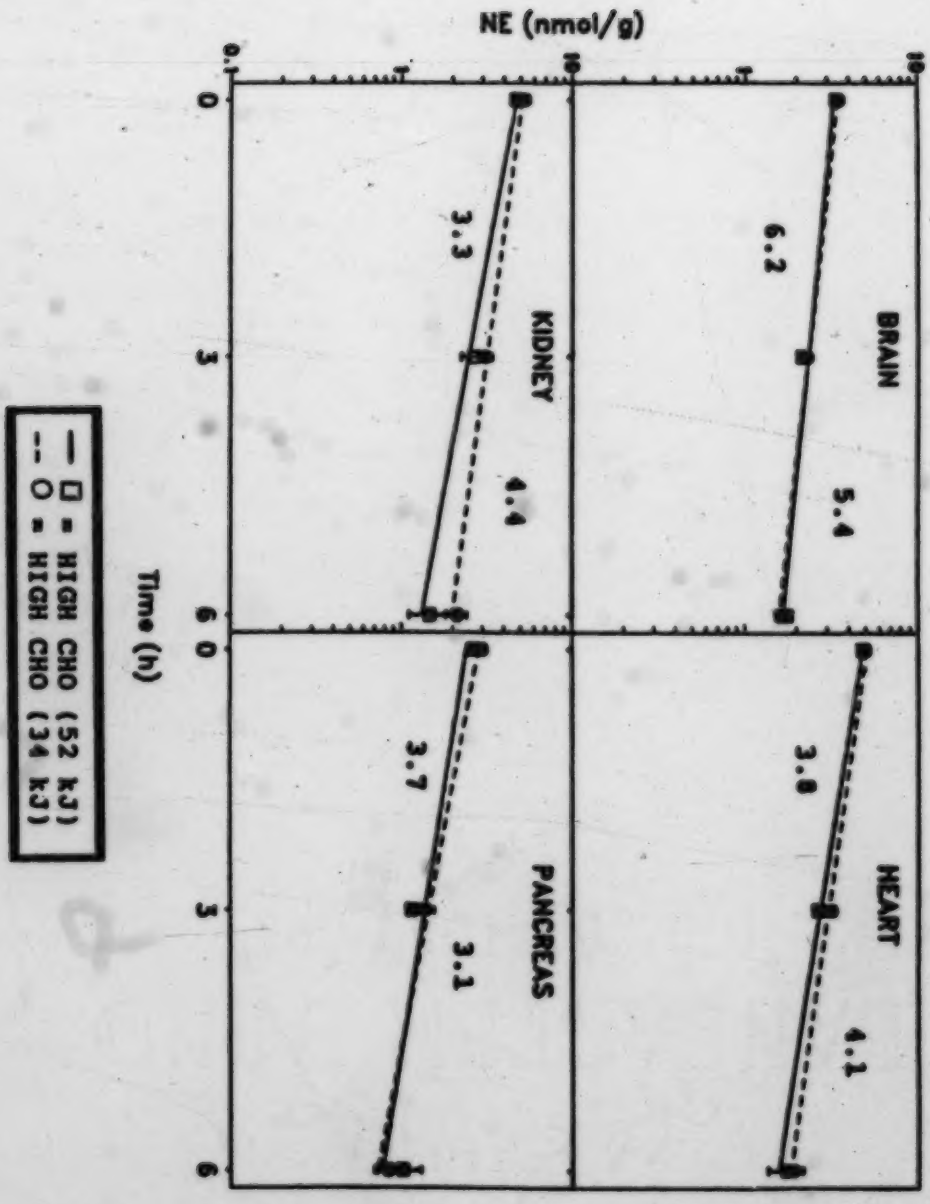
Group	Weight change (g/3d)
High CHO (52kJ)	-0.47 ± 0.11^{a1}
High CHO (34kJ)	-1.96 ± 0.14^{b}
High Fat (52kJ)	-0.47 ± 0.15^{a}
High Fat (34kJ)	-1.99 ± 0.16^{b}

[1] Values are means $\pm$ SEM; n=18; values with different superscripts are significantly different ($p < 0.01$).

Figure II-1. Disappearance of NE in mouse brain, heart, kidney and pancreas, after administration of α methyl-tyrosine in mice fed two levels of energy intake of a high carbohydrate diet: 52kJ (——) and 34kJ (---). Each point represents the mean ± SEM of 5-6 mice. The equations for the least squares fit of log transformed concentrations were:

BRAIN:	CHO (52kJ)	y=3.512-0.048x;	r=-0.85
	CHO (34kJ)	y=3.536-0.056x;	r=-0.90
HEART:	CHO (52kJ)	y=3.676-0.080x;	r=-0.87
	CHO (34kJ)	y=3.701-0.072x;	r=-0.88
KIDNEY:	CHO (52kJ)	y=3.672-0.092x;	r=-0.83
	CHO (34kJ)	y=3.708-0.068x;	r=-0.85
PANCREAS:	CHO (52kJ)	y=3.380-0.082x;	r=-0.79
	CHO (34kJ)	y=3.460-0.099x;	r=-0.87

The numbers shown are the half-times of disappearance of NE (in hours).


NE (nmol/g)
BRAIN
6.2
5.4
KIDNEY
3.3
4.4
HEART
3.8
4.1
PANCREAS
3.7
3.1
Time (h)
0
3
6
— □ = HIGH CHO (52 kJ)
-- ○ = HIGH CHO (34 kJ)

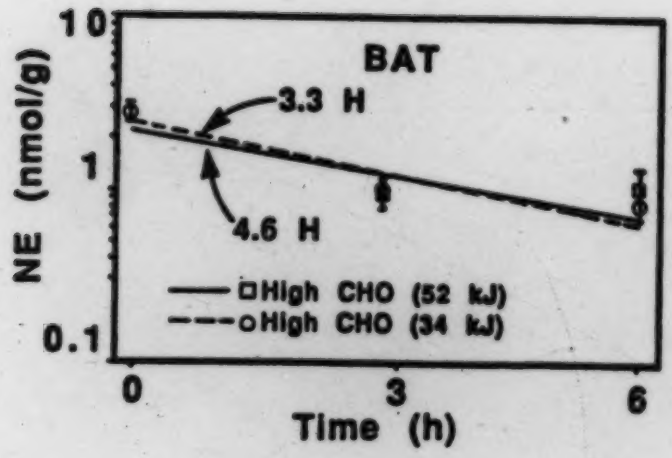


Figure II-2. Disappearance of NE in mouse IBAT after administration of α methyl-tyrosine in mice fed two levels of energy intake of a high carbohydrate diet: 52kJ (——) and 34kJ (---). Each point represents the mean ± SEM of 5-6 mice. The equations for the least squares fit of log transformed concentrations were:

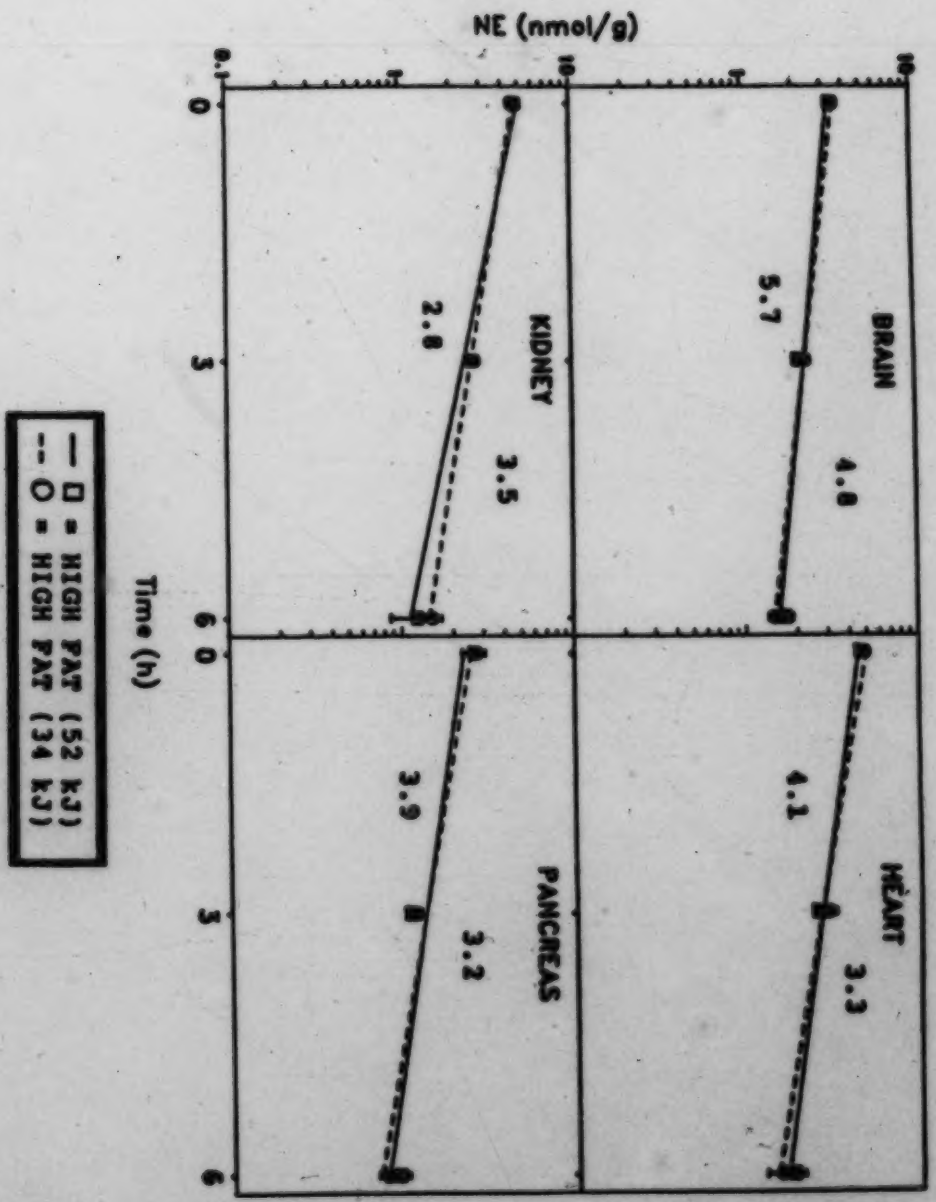
CHO (52kJ)	y=3.313-0.065x;	r=-0.72
CHO (34kJ)	y=3.369-0.092x;	r=-0.77

The numbers shown are the half-times of disappearance of NE (in hours).

Figure II-3. Disappearance of NE in mouse brain, heart, kidney and pancreas, after administration of α methyl-tyrosine in mice fed two levels of energy intake of a high fat diet: 52 kJ (——) and 34kJ (---). Each point represents the mean ± SEM of 5-6 mice. The equations for the least squares fit of log transformed concentrations were:

BRAIN:	FAT (52kJ)	y=3.513-0.053x;	r=-0.82
	FAT (34kJ)	y=3.547-0.063x;	r=-0.93
HEART:	FAT (52kJ)	y=3.647-0.072x;	r=-0.89
	FAT (34kJ)	y=3.693-0.091x;	r=-0.86
KIDNEY:	FAT (52kJ)	y=3.699-0.109x;	r=-0.85
	FAT (34kJ)	y=3.674-0.085x;	r=-0.90
PANCREAS:	FAT (52kJ)	y=3.357-0.078x;	r=-0.83
	FAT (34kJ)	y=3.401-0.093x;	r=-0.93

The numbers shown are the half-times of disappearance of NE (in hours).


NE (nmol/g)
BRAIN
5.7
4.0
KIDNEY
2.8
3.5
HEART
4.1
3.3
PANCREAS
3.9
3.2
0
3
6
Time (h)
— □ = HIGH FAT (52 kJ)
-- ○ = HIGH FAT (34 kJ)

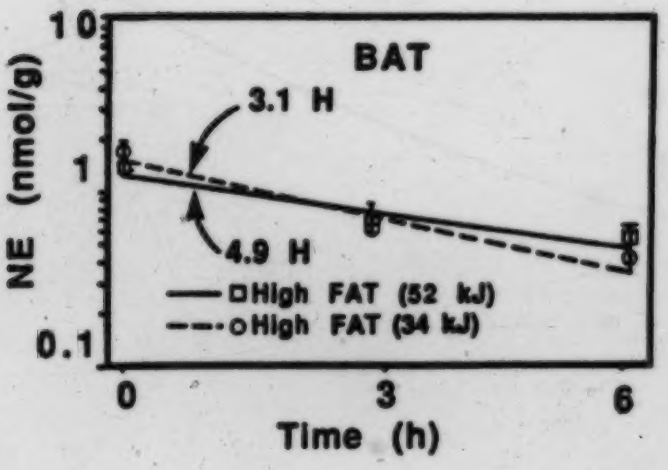


Figure II-4. Disappearance of NE in mouse IBAT after administration of α methyl-tyrosine in mice fed two levels of energy intake of a high fat diet: 52kJ (——) and 34kJ (---). Each point represents the mean ± SEM of 5-6 mice. The equations for the least squares fit of log transformed concentrations were:

FAT (52kJ) y=3.087-0.062x; r=-0.71
FAT (34kJ) y=3.170-0.099x; r=-0.80

The numbers shown are the half-times of disappearance of NE (in hours).

Experiment 2. The restricted mice were fed their daily intake (34 kJ) as 2 equal meals presented at the beginning and the middle of the dark period to eliminate the fasting effect of normal energy restriction. When mice were allowed ad libitum access to the high carbohydrate diet, intake averaged 2.90±0.11g over the experimental period, 37% higher than the intake of these "meal-fed" restricted mice (Table II-5). These levels of intake produced the expected increase in weight in the ad libitum fed mice, while the "meal-fed" restricted mice lost weight (Table II-5).

In spite of the elimination of the fasting effect of deprivation, both endogenous NE concentration and fractional NE turnover were unaffected by the restriction of energy intake (Figures II:5-6, Table II-6). Fractional turnover, however, tended to be lower in heart (by 15%), kidney (20%), and pancreas (12%), and higher in brain (by 28%) and IBAT (18%) in the "meal-fed" restricted mice, compared to their ad libitum fed controls. In addition, the pancreas and IBAT of the ad libitum fed mice were 12% and 19% heavier, respectively, than the "meal-fed" restricted animals (Table II-6).

The inability of restriction per se to affect turnover suggests that the predominant effects of energy on NE turnover may be due to the fasting that accompanies normal energy restriction.

Experiment 2
Table II-5.

Effect of energy restriction[1] on food intake and weight change

Group	Intake (g/d)	Intake (kJ/d)	Weight change (g/3d)
High CHO (ad lib)	2.90±0.11[a2]	46.16±1.70[a]	+0.36±0.07[a]
High CHO (2 x 17kJ)	2.12±0.00[b]	33.75±0.00[b]	-1.24±0.11[b]

[1] Animals were meal-fed the previously determined restricted intake divided into two equal meals presented at the beginning and middle of the dark period.

[2] Values are means ± SEM; n=18; values within columns with a different superscript are significantly different ($p < 0.001$).

Experiment 2
Table II-6.

Norepinephrine(NE) turnover in ad libitum fed or "meal-fed" restricted mice

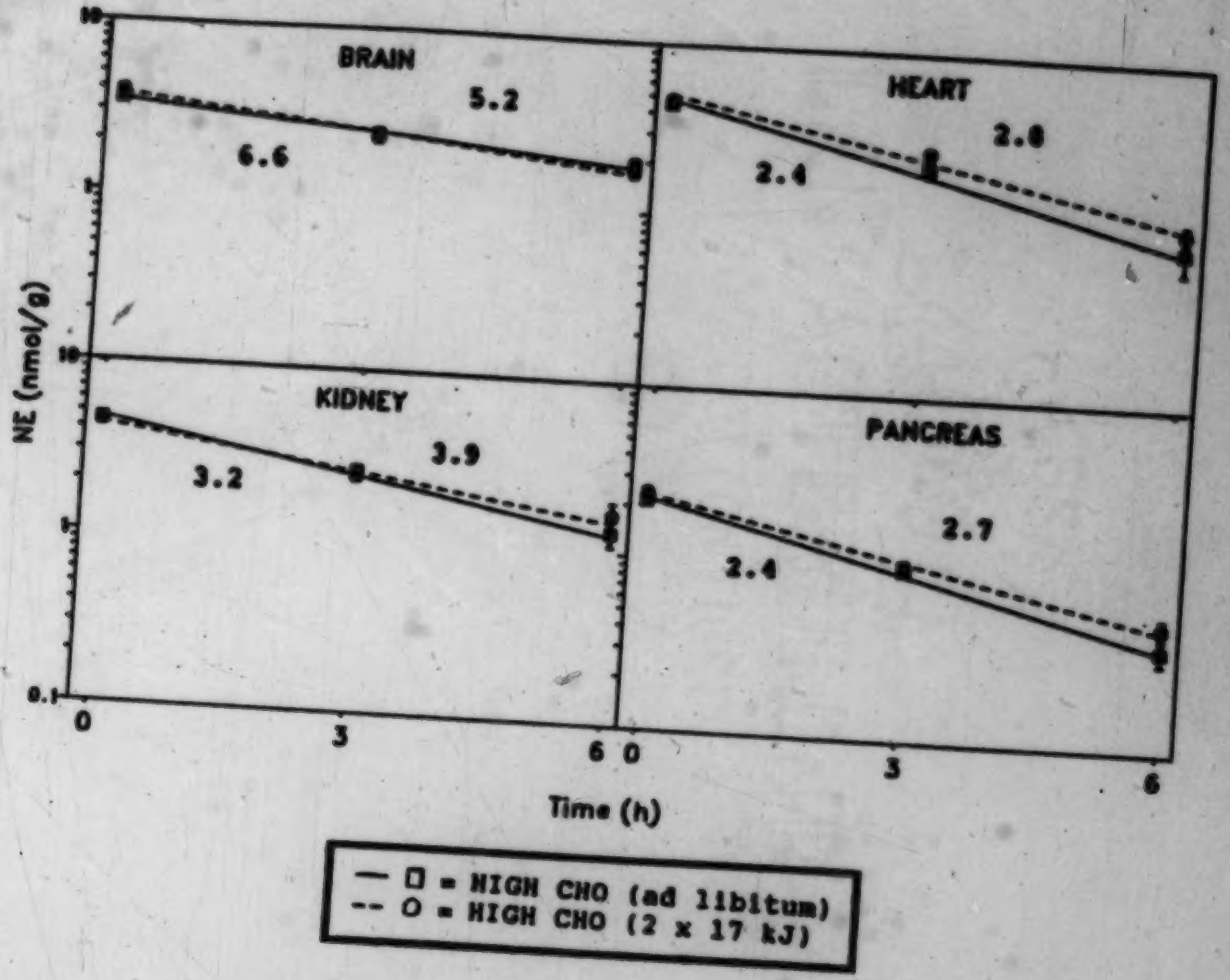
Group	Weight (mg)	NE (nmol/g)	Fractional NE (k) turnover (%h)	Calculated turnover rate (nmol/g/h)
		BRAIN		
High CHO (ad lib)	197.3±3.0[1]	3.51±0.18	10.5±0.01	0.361
High CHO (2 x 17kJ)	205.3±3.9	3.88±0.23	13.4±0.01	0.499
		HEART		
High CHO (ad lib)	85.7±2.0	4.52±0.16	29.5±0.02	1.400
High CHO (2 x 17kJ)	80.4±1.9	4.82±0.17	25.2±0.01	1.286
		KIDNEY		
High CHO (ad lib)	95.4±2.1	4.55±0.10	21.9±0.01	1.018
High CHO (2 x 17kJ)	89.8±2.3	4.42±0.16	17.6±0.01	0.754
		PANCREAS		
High CHO (ad lib)	107.3±3.9[a]	2.19±0.07	29.4±0.01	0.663
High CHO (2 x 17kJ)	96.1±2.7[b]	2.45±0.13	25.8±0.01	0.612
		IBAT		
High CHO (ad lib)	167.6±10.9[a]	3.05±0.20	23.2+0.02	0.567
High CHO (2 x 17kJ)	141.5± 5.5[b]	3.46±0.32	27.3+0.01	0.778

[1] Values are means ± SEM; n=18; values within columns with a different superscript are significantly different ($p < 0.05$).

Figure II-5. Disappearance of NE in mouse brain, heart, kidney and pancreas, after administration of α methyl-tyrosine in mice fed a high carbohydrate diet ad libitum (——) or restricted and meal-fed 34kJ as two daily meals (---).
Each point represents the mean ± SEM of 5-6 mice.
The equations for the least squares fit of log transformed concentrations were:

BRAIN:	CHO (ad lib)	y=3.535-0.046x; r=-0.82
	CHO (2x17kJ)	y=3.570-0.057x; r=-0.88
HEART:	CHO (ad lib)	y=3.677-0.128x; r=-0.92
	CHO (2x17kJ)	y=3.708-0.109x; r=-0.96
KIDNEY:	CHO (ad lib)	y=3.667-0.095x; r=-0.91
	CHO (2x17kJ)	y=3.632-0.076x; r=-0.87
PANCREAS:	CHO (ad lib)	y=3.353-0.126x; r=-0.96
	CHO (2x17kJ)	y=3.376-0.112x; r=-0.95

The numbers shown are the half-times of disappearance of NE (in hours).


BRAIN
5.2
6.6
HEART
2.8
2.4
KIDNEY
3.9
3.2
PANCREAS
2.7
2.4
NE (nmol/g)
10
1
0.1
0
3
6
0
3
6
Time (h)
□ = HIGH CHO (ad libitum)
○ = HIGH CHO (2 x 17 kJ)

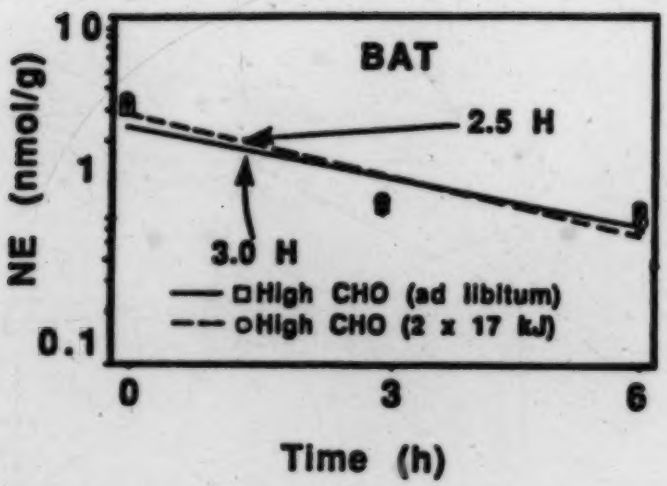


Figure II-6. Disappearance of NE in mouse IBAT after administration of α methyl-tyrosine in mice fed a high carbohydrate diet ad libitum (——) or restricted and meal-fed 34kJ as two daily meals (---). Each point represents the mean ± SEM of 5-6 mice. The equations for the least squares fit of log transformed concentrations were:

CHO (ad lib)	y=3.389-0.101x;	r=-0.83
CHO (2x17kJ)	y=3.455-0.118x;	r=-0.87

The numbers shown are the half-times of disappearance of NE (in hours).

Experiment 3. When mice were fed the restricted intake (34kJ) presented as a single meal, or were allowed ad libitum access to the high carbohydrate diet, the restricted mice lost and ad libitum fed mice gained weight (Table II-7). Intake of the ad libitum fed group was 57% higher than that of the restricted group. This restriction with fasting resulted in a significant 38% lower NE turnover in the heart alone of the restricted mice compared to their ad libitum fed controls (Figures II:7-8, Table II-8). In addition, turnover in the remaining organs tended to be lower in the kidney (by 22%), pancreas (23%) and IBAT (25%), and higher in the brain (17%) of the restricted mice, although not significantly so. Cardiac NE concentration was unaffected by this restriction, however, the hearts of the ad libitum fed mice were 10% heavier than those of the restricted animals (Table II-8). Pancreatic NE concentration was affected by restriction, restricted mice having a 20% higher concentration of NE than their ad libitum fed controls (Table II-8). The weights of the pancreas and IBAT of the ad libitum fed mice were 21% and 32% higher, respectively, than those of the restricted mice (Table II-8).

Experiment 3
Table II-7.

Effect of energy restriction[1] on food intake and weight change

Group	Intake (g/d)	Intake (kJ/d)	Weight change (g/3d)
High CHO (ad lib)	3.33±0.09[a2]	53.01±1.39[a]	+0.82±0.12[a]
High CHO (34kJ)	2.12±0.00[b]	33.75±0.00[b]	-1.04±0.09[b]

[1] Animals were fed the previously determined restricted intake as a single meal.

[2] Values are means + SEM; n=17-18; values within columns with a different superscript are significantly different ($p < 0.001$).

Experiment 3
Table II-8.

Norepinephrine(NE) turnover in ad libitum fed or restricted mice

Group	Weight (mg)	NE (nmol/g)	Fractional NE (K) turnover (%h)	Calculated turnover rate (nmol/g/h)
		BRAIN		
High CHO (ad lib)	202.4±2.9[1]	3.61±0.07	10.9±0.00	0.385
High CHO (34kJ)	199.6±3.3	3.88±0.13	12.7±0.00	0.481
		HEART		
High CHO (ad lib)	87.8±1.6[a]	4.38±0.14	38.5±0.03[a]	1.930
High CHO (34kJ)	80.8±1.8[b]	4.64±0.20	23.7±0.01[b]	1.180
		KIDNEY		
High CHO (ad lib)	94.2±1.4	4.00±0.02	25.1±0.02	1.068
High CHO (34kJ)	89.3±2.1	4.12±0.15	19.6±0.01	0.805
		PANCREAS		
High CHO (ad lib)	103.5±3.1[a]	1.86±0.06[a]	27.2±0.01	0.526
High CHO (34kJ)	85.3±3.2[b]	2.24±0.11[b]	20.9±0.01	0.447
		IBAT		
High CHO (ad lib)	178.1±6.8[a]	2.61±0.12	29.6±0.01	0.695
High CHO (34kJ)	134.9±6.9[b]	2.98±0.19	22.1±0.01	0.526

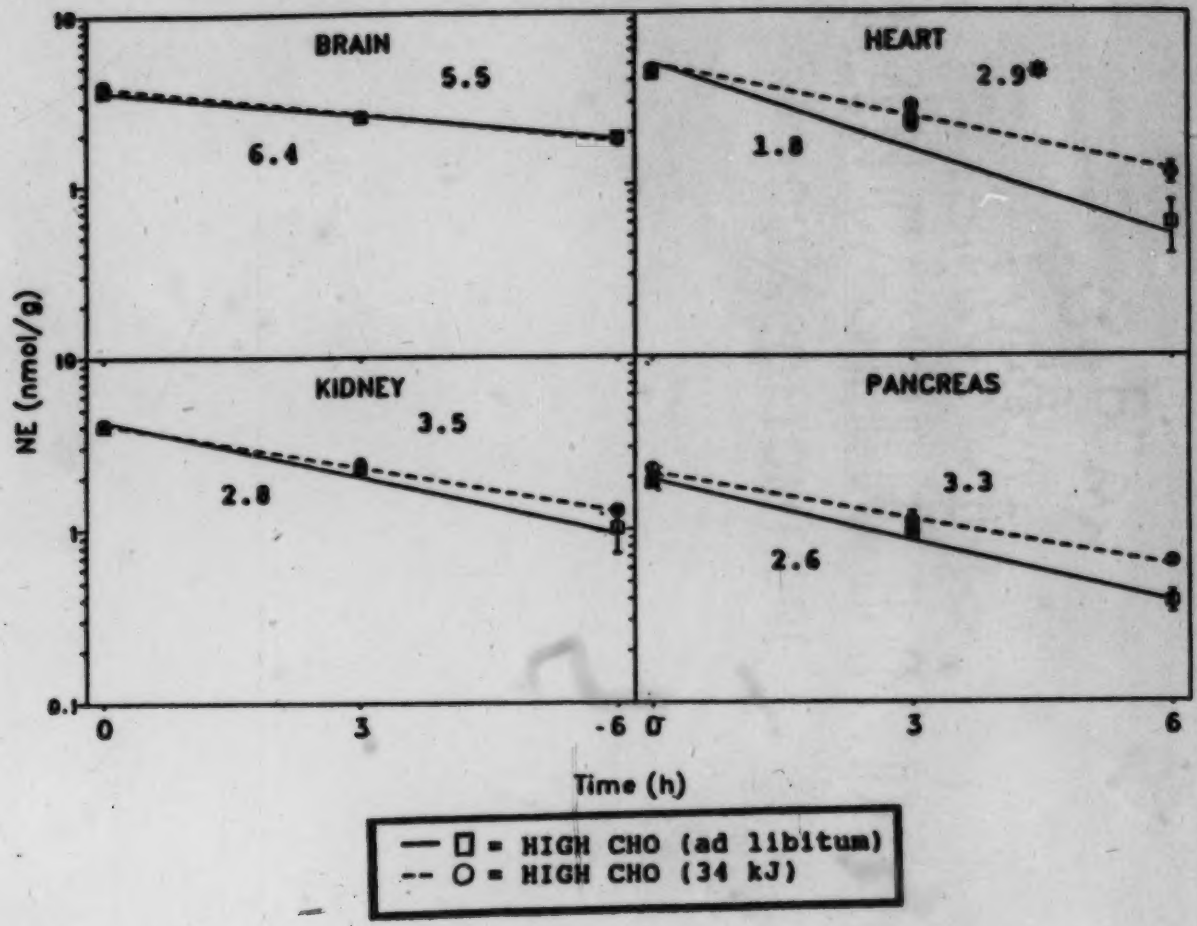
[1] Values are means ± SEM; n=18; values within columns with a different superscript are significantly different ($p < 0.05$).

Figure II-7. Disappearance of NE in mouse brain, heart, kidney and pancreas, after administration of α methyl-tyrosine in mice fed a high carbohydrate diet ad libitum (——) or restricted to 34kJ (---) fed as a single daily meal.
Each point represents the mean ± SEM of 5-6 mice.
The equations for the least squares fit of log transformed concentrations were:

BRAIN:	CHO (ad lib)	y=3.550-0.047x;	r=-0.92
	CHO (34kJ)	y=3.580-0.055x;	r=-0.94
HEART:	CHO (ad lib)	y=3.677-0.141x;	r=-0.91
	CHO (34kJ)	y=3.698-0.103x;	r=-0.93
KIDNEY:	CHO (ad lib)	y=3.629-0.109x;	r=-0.87
	CHO (34kJ)	y=3.613-0.085x;	r=-0.93
PANCREAS:	CHO (ad lib)	y=3.286-0.118x;	r=-0.95
	CHO (34kJ)	y=3.329-0.091x;	r=-0.92

The numbers shown are the half-times of disappearance of NE (in hours).

*Slope of the regression line is significantly different from control ($p < 0.05$).


BRAIN
5.5
6.4
HEART
2.9*
1.8
KIDNEY
3.5
2.8
PANCREAS
3.3
2.6
NE (nmol/g)
10
1
0.1
0
3
-6
0
3
6
Time (h)
— □ = HIGH CHO (ad libitum)
-- ○ = HIGH CHO (34 kJ)

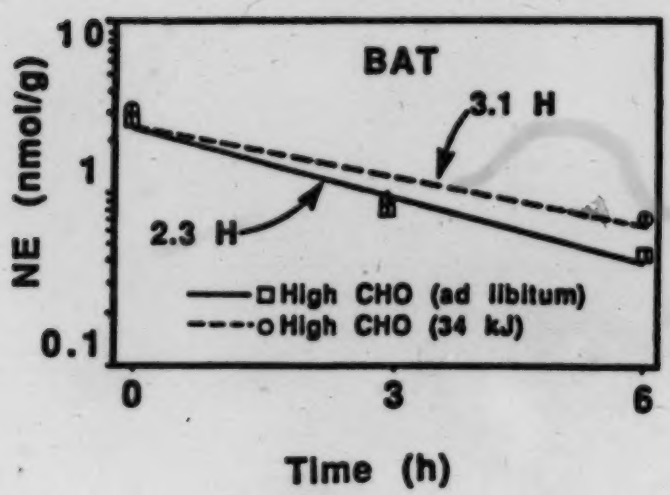


Figure II-8. Disappearance of NE in mouse IBAT after administration of α methyl-tyrosine in mice fed a high carbohydrate diet ad libitum (——) or restricted to 34kJ fed as a single daily meal (---). Each point represents the mean ± SEM of 5-6 mice. The equations for the least squares fit of log transformed concentrations were:

CHO (ad lib)	y=3.370-0.129x;	r=-0.90
CHO (34kJ)	y=3.376-0.096x;	r=-0.83

The numbers shown are the half-times of disappearance of NE (in hours).

Experiment 4. Because the ad libitum intakes in Exp. 2 and 3 varied, the degree of restriction also varied: "meal-fed" restricted (Exp. 2) and restricted (Exp. 3) mice were fed 73% and 64%, respectively, of their corresponding ad libitum intakes (Tables II-5, II-7). It was therefore difficult to determine whether the decrease in cardiac NE turnover observed when the restricted intake was presented as a single meal (Table II-8) was in fact due to fasting or to the relatively greater degree of energy restriction in Exp. 3, due to the differences in ad libitum intake. Notably, fractional NE turnover in the heart of mice fed the high carbohydrate diet with an ad libitum intake of 53 kJ (Exp. 3) (Table II-7) is 30% higher than that in the heart of mice consuming an ad libitum intake of 46 kJ (Exp. 2) (Table II-5).

Mice were fed the high carbohydrate diet ad libitum, or restricted to 24 kJ/day (a 30% lower intake than that provided with previous restricted intakes of 34 kJ/day) presented as either one or two daily meals. Ad libitum fed mice had an intake almost twice that of the restricted animals (Table II-9). Although "meal-fed" restricted and restricted mice received identical total energy intakes, weight loss in the restricted mice was 33% greater than that in the "meal-fed" restricted mice (Table II-9). Conversely, the ad libitum fed mice gained weight (Table II-9).

Fractional NE turnover was again lower, by 46%, in the heart only, of the restricted mice compared to their ad

libitum fed counterparts, when the restricted intake was presented as a single meal (Figures II:9-10, Table II-10). Conversely, when an identical intake was presented as two daily meals, fractional NE turnover did not differ from that of ad libitum fed counterparts in any organ (Figures II:11-12, Table II-10).

Endogenous NE concentration was unaffected by either restriction or "meal-feeding" restriction, except in heart and pancreas where concentration was higher when either type of restricted intake was fed, compared to the ad libitum fed controls (Table II-10). With the exception of brain, the weight of all organs was lower in both groups of mice fed restricted intakes, compared to the ad libitum fed mice.

Experiment 4
Table II-9.

Effect of energy restriction on food intake and weight change

Group	Intake (g/d)	Intake (kJ/d)	Weight change (g/3d)
High CHO (ad lib)	2.91±0.06[a1]	46.33±1.0[a]	+0.62±0.12[a]
High CHO (2 x 12kJ)	1.50±0.00[b]	23.88±0.0[b]	-1.32±0.14[b]
High CHO (24kJ)	1.50±0.00[b]	23.88±0.0[b]	-1.76±0.07[c]

[1] Values are means ± SEM; n=18; values within columns with a different superscript are significantly different ($p < 0.01$).

Experiment 4
Table II-10.

Norepinephrine(NE) turnover in ad libitum fed, "meal-fed" restricted, or restricted mice

Group	Weight (mg)	NE (nmol/g)	Fractional NE (k) turnover (%h)	Calculated turnover rate (nmol/g/h)
		BRAIN		
High CHO (ad lib)	207.1±2.6[1]	3.36±0.08	10.1±0.01	0.327
High CHO (2 x 12kJ)	200.6±4.1	3.46±0.10	10.7±0.00	0.367
High CHO (24kJ)	203.9±4.3	3.62±0.16	13.1±0.01	0.466
		HEART		
High CHO (ad lib)	80.0±1.6[a]	5.02±0.19[a]	28.5±0.02[a]	1.565
High CHO (2 x 12kJ)	73.6±1.3[b]	5.63±0.10[b]	22.6±0.01[a]	1.437
High CHO (24kJ)	76.6±0.7[b]	5.70±0.22[b]	15.5±0.01[b]	0.890
		KIDNEY		
High CHO (ad lib)	100.3±2.2[a]	4.63±0.10	19.0±0.01	0.886
High CHO (2 x 12kJ)	86.7±2.4[b]	4.78±0.15	15.4±0.01	0.768
High CHO (24kJ)	92.6±1.9[ab]	5.14±0.15	15.4±0.01	0.764
		PANCREAS		
High CHO (ad lib)	95.1±2.6[a]	2.38±0.08[a]	20.8±0.01	0.468
High CHO (2 x 12kJ)	82.2±2.8[b]	2.91±0.15[b]	19.4±0.01	0.563
High CHO (24kJ)	82.2±2.6[b]	2.88±0.13[b]	20.4±0.01	0.565
		IBAT		
High CHO (ad lib)	136.7±8.2[a]	3.51±0.21	25.4±0.01	0.745
High CHO (2 x 12kJ)	105.8±6.8[b]	3.74±0.35	21.3±0.01	0.702
High CHO (24kJ)	103.1±6.0[b]	4.38±0.28	23.8±0.01	0.897

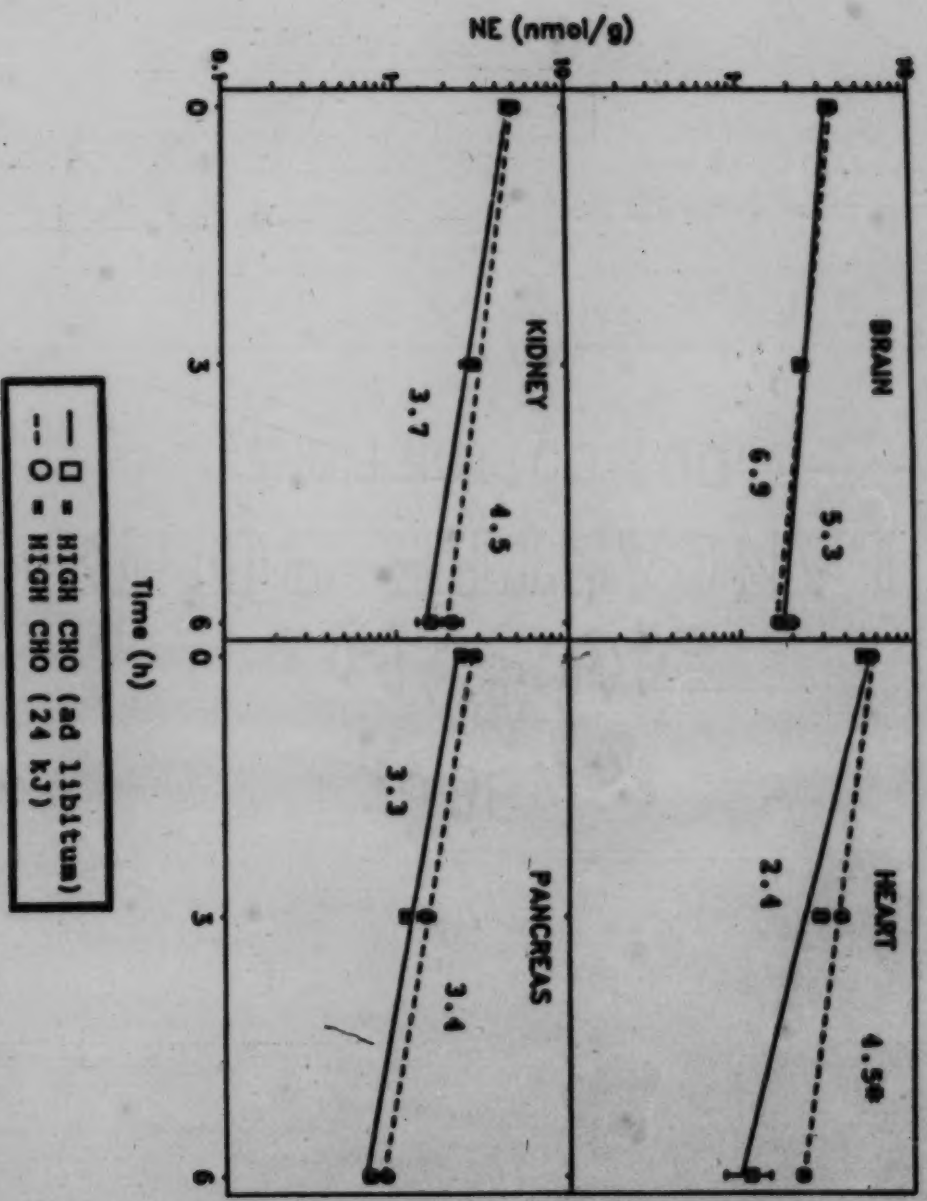
[1] Values are means ± SEM; n=18; values within columns with a different superscript are significantly different ($p < 0.01$).

Figure II-9. Disappearance of NE in mouse brain, heart, kidney and pancreas, after administration of α methyl-tyrosine in mice fed a high carbohydrate diet ad libitum (——) or restricted to 24 kJ fed as a single daily meal (---).
Each point represents the mean ± SEM of 5-6 mice.
The equations for the least squares fit of log transformed concentrations were:

BRAIN:	CHO (ad lib)	y=3.512-0.044x; r=-0.82
	CHO (24kJ)	y=3.550-0.057x; r=-0.93
HEART:	CHO (ad lib)	y=3.730-0.124x; r=-0.84
	CHO (24kJ)	y=3.760-0.067x; r=-0.93
KIDNEY:	CHO (ad lib)	y=3.670-0.083x; r=-0.87
	CHO (24kJ)	y=3.696-0 067x; r=-0.90
PANCREAS:	CHO (ad lib)	y=3.353-0.090x; r=-0.95
	CHO (24kJ)	y=3.443-0.088x; r=-0.93

The numbers shown are the half-times of disappearance of NE (in hours).

*Slope of the regression line is significantly different from control ($p < 0.01$).


NE (nmol/g)
BRAIN
6.9
5.3
KIDNEY
3.7
4.5
HEART
2.4
4.5*
PANCREAS
3.3
3.4
0
3
6
Time (h)
— □ = HIGH CHO (ad libitum)
-- O = HIGH CHO (24 kJ)

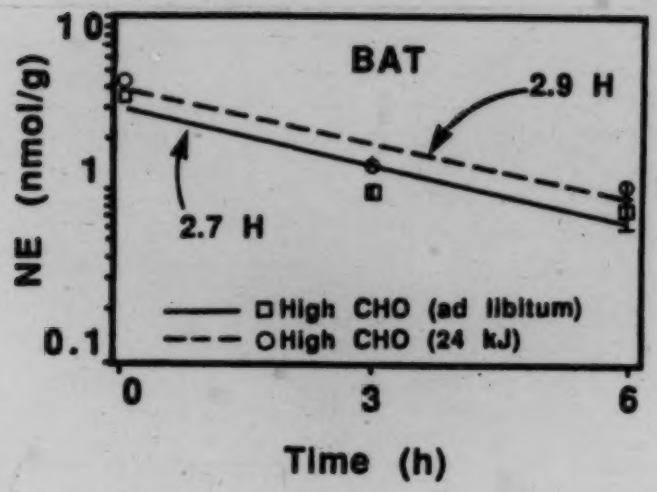


Figure II-10. Disappearance of NE in mouse IBAT after administration of α methyl-tyrosine in mice fed a high carbohydrate diet ad libitum (——) or restricted to 24 kJ fed as a single daily meal(---). Each point represents the mean ± SEM of 5-6 mice. The equations for the least squares fit of log transformed concentrations were:

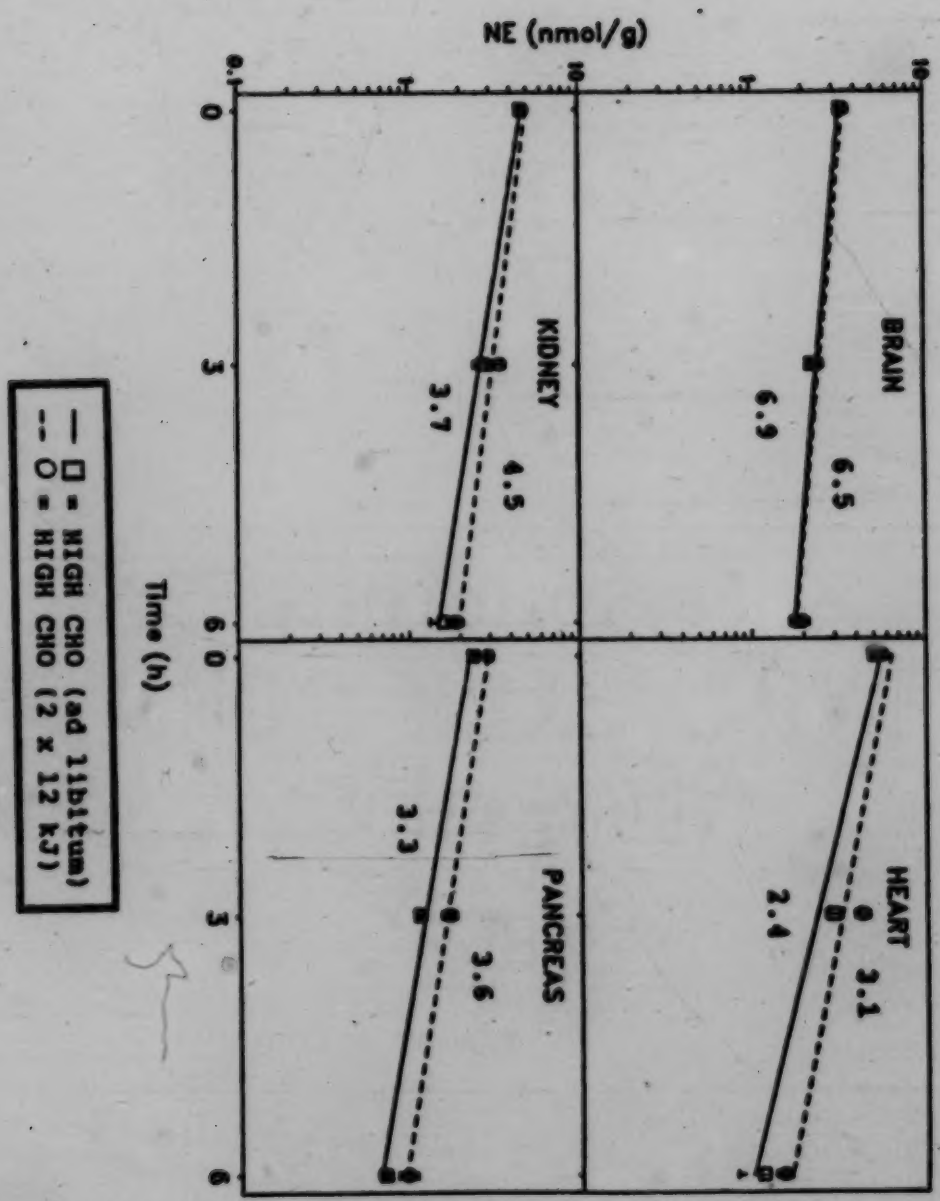
CHO (ad lib)	y=3.468-0.110x;	r=-0.89
CHO (24kJ)	y=3.577-0.103x;	r=-0.91

The numbers shown are the half-times of disappearance of NE (in hours).

Figure II-11. Disappearance of NE in mouse brain, heart, kidney and pancreas, after administration of α methyl-tyrosine in mice fed a high carbohydrate diet ad libitum (——) or restricted and "meal-fed" 24kJ as two daily meals (---).
Each point represents the mean ± SEM of 5-6 mice.
The equations for the least squares fit of log transformed concentrations were:

BRAIN:	CHO (ad lib)	y=3.512-0.044x;	r=-0.82
	CHO (2x12kJ)	y=3.535-0.047x;	r=-0.95
HEART:	CHO (ad lib)	y=3.739-0.124x;	r=-0.84
	CHO (2x12kJ)	y=3.806-0.098x;	r=-0.91
KIDNEY:	CHO (ad lib)	y=3.670-0.083x;	r=-0.87
	CHO (2x12kJ)	y=3.697-0.067x;	r=-0.91
PANCREAS:	CHO (ad lib)	y=3.353-0.090x;	r=-0.95
	CHO (2x12kJ)	y=3.464-0.084x;	r=-0.91

The numbers shown are the half-times of disappearance of NE (in hours).


NE (nmol/g)
BRAIN
6.9
6.5
KIDNEY
3.7
4.5
HEART
2.4
3.1
PANCREAS
3.3
3.6
Time (h)
0
3
6
— □ = HIGH CHO (ad libitum)
-- ○ = HIGH CHO (2 x 12 kJ)

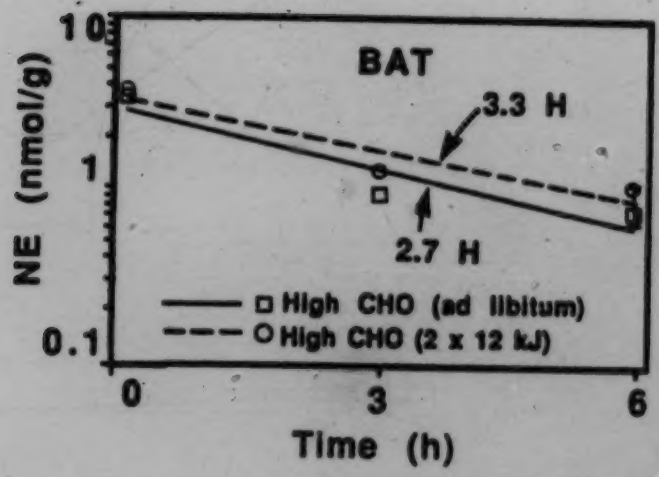


Figure II-12. Disappearance of NE in mouse IBAT after administration of α methyl-tyrosine in mice fed a high carbohydrate diet ad libitum (——) or restricted and "meal-fed" 24kJ as two daily meals (---). Each point represents the mean ± SEM of 5-6 mice.The equations for the least squares fit of log transformed concentrations were:

CHO (ad lib)	y=3.468-0.110x;	r=-0.89
CHO (2x12kJ)	y=3.517-0.092x;	r=-0.89

The numbers shown are the half-times of disappearance of NE (in hours).

Experiment 5. The dietary restrictions in Exp. 3 and 4 lowered cardiac NE turnover and provided a new dietary situation to test possible mechanisms for diet-induced changes in sympathetic activity. The effects of six dietary treatments (a high carbohydrate diet, fed ad libitum or restricted to 24 kJ provided as one or two daily meals; a high fat diet fed ad libitum or restricted to 24 kJ provided as a single daily meal; or a high protein diet fed ad libitum) on food intake and weight change are shown in Table II-11, and on serum glucose, total free fatty acids, insulin and large neutral amino acids are shown in Tables II-12 and II-13.

The ad libitum intakes, in grams, of the three experimental diets ranked in descending order were as follows: (1) high carbohydrate; (2) high protein; and (3) high fat (Table II-11). However, because of the differences in the energy densities of the three diets, mice fed the high carbohydrate or the high fat diets ad libitum had the greatest energy intakes of all treatment groups (Table II-11). In addition, mice fed the high protein diet ad libitum had a significantly lower energy intake than the other ad libitum fed mice (Table II-11). Restricted intakes of the high carbohydrate and the high fat groups were 52% and 54% of their corresponding ad libitum intakes. Body weight gains were similar in mice fed the ad libitum intake of the high carbohydrate and the the high fat diets (Table II-11). Mice fed the high protein diet ad libitum had a negligible loss

of body weight, while mice fed 24 kJ of either the high carbohydrate or the high fat lost more weight when the restricted intakes were presented as single meals than as two meals (Table II-11).

Serum glucose was highest in mice fed the high fat diet ad libitum and lowest in mice fed the restricted intakes, regardless of diet composition or number of daily meals (Table II-12). Mice fed the high protein diet ad libitum had serum glucose concentrations similar to those observed in the restricted mice and in those fed the high carbohydrate diet ad libitum.

FFA concentration was highest in the mice fed restricted intakes independent of diet composition or number of daily meals (Table II-12). When mice were fed the high fat diet ad libitum, serum FFA concentrations were only slightly below those observed in those fed 24 kJ of the same diet. Serum FFA concentrations were lowest in mice fed the high protein or the high carbohydrate diets ad libitum (Table II-12).

Serum insulin was unaffected by dietary treatment (Table II-12). However, restriction of the high fat diet produced a non significant 21% lower serum insulin, while a similar restriction of the high carbohydrate diet produced, respectively, 10% and 8% higher serum insulin concentration when the restricted intake was fed as one or two daily meals (Table II-11).

Serum tyrosine concentrations were highest in the ad libitum fed mice, regardless of diet composition (Table II-

13). Mice fed the high fat diet ad libitum, had respectively, 1.5-, 1.9- or 2.4-fold higher serum tyrosine concentrations than those fed restricted intakes of either the high fat diet, or the high carbohydrate diet as one daily meal or two daily meals (Table II-13).

Ad libitum consumption of the high protein diet produced a higher sum of other NAA in serum when compared to all other diets (Table II-13). Mice fed 24 kJ of either the high fat or the high carbohydrate diet had serum totals of other NAA which were not significantly different from their ad libitum fed counterparts. However, in mice "meal-fed" the restricted intake of the high carbohydrate diet, the sum of the other NAA in serum was lower than those observed in mice fed the same diet ad libitum.

Because the NAA concentrations in the high protein group were so high, the tyrosine: other NAA ratio in these mice was lower than the ratio in mice on all other dietary regimens (Table II-13). Mice fed the high carbohydrate or the high fat diet either ad libitum or restricted to 24 kJ fed as a single daily meal, had similar tyrosine: other NAA ratios. However, when the restricted intake of the high carbohydrate diet was fed as two daily meals, the ratio was significantly lower than in mice fed ad libitum intakes of either the high carbohydrate or high fat diet, and only slightly less than in the other restricted groups.

The relationship between serum concentrations of glucose, FFA, insulin, tyrosine, other NAA and the tyrosine:

other NAA ratio and corresponding fractional NE turnover values is reported in Table II-14. There was no significant relationship between fractional NE turnover rate and any of these variables ($p >> 0.05$). Fractional NE turnover rate was significantly related to energy intake in situations of sympathetic activation. However, the relationship is not significant when the influence of body weight is eliminated by the determination of the partial correlation coefficient (Table II-15).

Experiment 5
Table II-11.

Effect of diet and energy restriction on food intake and weight change

Group	Intake (g/d)	Intake (kJ/d)	Weight change (g/3d)
High CHO (ad lib)	2.91±0.06[a1]	46.33±1.0[a]	+0.62±0.12[a]
High CHO (2 x 12kJ)	1.50±0.00[d]	23.88±0.0[c]	-1.32±0.14[c]
High CHO (24kJ)	1.50±0.00[d]	23.88±0.0[c]	-1.76±0.07[d]
High Fat (ad lib)	2.12±0.05[c]	44.52±1.0[a]	+0.50±0.12[a]
High Fat (24kJ)	1.14±0.00[e]	23.88±0.0[c]	-1.80±0.09[d]
High Protein (ad lib)	2.30±0.07[b]	40.15±1.2[b]	-0.02±0.10[b]

[1] Values are means ± SEM; n=15-18; values within columns with a different superscript are significantly different ($p < 0.01$).

Experiment 5
Table II-12.

Effect of diet on serum glucose, free fatty acids(FFA) and insulin concentrations

Group	Serum Glucose (mmol/dL)	Serum FFA (umol/dL)	Serum Insulin (mU/L)
High CHO (ad lib)	10.37 ± 0.4^{abl}	41.5 ± 4.4^{c}	9.5 ± 0.9
High CHO (2 x 12kJ)	8.41 ± 0.2^{c}	58.2 ± 5.8^{a}	10.3 ± 0.9
High CHO (24kJ)	8.45 ± 0.3^{c}	58.6 ± 2.8^{a}	10.4 ± 1.1
High Fat (ad lib)	11.27 ± 0.3^{a}	45.8 ± 3.0^{bc}	9.1 ± 1.3
High Fat (24kJ)	9.30 ± 0.4^{c}	55.0 ± 4.0^{ab}	7.2 ± 0.9
High Protein (ad lib)	9.61 ± 0.2^{bc}	39.4 ± 3.8^{c}	9.5 ± 0.4

[1] Values are means ± SEM; n=6; values within columns with a different superscript are significantly different ($p < 0.01$).

Experiment 5
Table II-13.

Effect of diet on serum tyrosine, other NAA[1] and tyrosine/other NAA

Group	Tyrosine (umol/dL)	Other NAA (umol/dL)	Tyrosine/ Other NAA
High CHO (ad lib)	6.12 ± 0.46^{ab}[2]	40.46 ± 1.83^{bc}	0.15 ± 0.01^{a}
High CHO (2 x 12kJ)	2.90 ± 0.32^{d}	25.02 ± 2.99^{def}	0.12 ± 0.00^{bc}
High CHO (24kJ)	3.68 ± 0.37^{cd}	26.69 ± 2.04^{cde}	0.14 ± 0.01^{ab}
High Fat (ad lib)	6.96 ± 1.16^{a}	41.47 ± 3.49^{b}	0.16 ± 0.01^{a}
High Fat (24kJ)	4.58 ± 0.22^{bcd}	32.40 ± 1.86^{bcd}	0.14 ± 0.01^{ab}
High Protein (ad lib)	5.85 ± 0.28^{abc}	87.37 ± 7.37^{a}	0.07 ± 0.01^{d}

[1] Other NAA: Valine, leucine, isoleucine, phenylalanine and tryptophan.

[2] Values are means ± SEM; n=6; values within columns with a different superscript are significantly different ($p < 0.01$).

Experiment 5
Table II-14.

Relationship between serum glucose, FFA, insulin, tyrosine, other NAA and tyrosine/other NAA[1] and fractional NE turnover[2]

Group	High CHO (ad lib)	High CHO (2 x 12kJ)	High CHO (24kJ)	High Protein (ad lib)	r[3]
Fractional NE turnover (%h)	28.5	22.6	15.5	14.7	
Glucose (mmol/dL)	10.37	8.41	8.45	9.61	+0.453
FFA (umol/dL)	41.5	58.2	58.6	39.4	-0.161
Insulin (mU/L)	9.5	10.3	10.4	9.5	-0.263
Tyrosine (umol/dL)	6.12	2.90	3.68	5.85	+0.192
Other NAA (umol/dL)	40.46	25.02	26.69	87.37	-0.409
Tyrosine / Other NAA	0.15	0.12	0.14	0.07	+0.619

[1] Other NAA: Valine, leucine, isoleucine, phenylalanine and tryptophan.

[2] Turnover values determined from Exp. 4 and reference 11 for high protein.

[3] The simple correlation coefficient.

Experiment 5
Table II-15.

Relationship between energy intake, body weight and fractional NE turnover in heart

	Energy intake[1] kJ/d)	Body weight[2] (g)	Fractional NE turnover[3] (%h)
High CHO (ad lib) [Exp. 1]	46.16	16.25	29.5
High CHO (2x17kJ) [Exp. 1]	33.75	14.46	25.2
High CHO (ad lib) [Exp. 2]	53.01	16.52	38.5
High CHO (ad lib) [Exp. 3]	46.32	16.66	28.5
High CHO (2x12kJ) [Exp. 3]	23.88	14.73	22.6

r^{12} = +0.891; $p < 0.05$ [simple correlation coefficient]
r^{13} = +0.890; $p < 0.05$ [simple correlation coefficient]
r^{23} = +0.733; $p > 0.05$ [simple correlation coefficient]

$r^{13.2}$ = +0.767; $p > 0.05$ [partial correlation coefficient]

D. Discussion and conclusions

The results of this study indicate that food deprivation, and not energy intake per se, is the predominant factor in the organ-specific suppression of NE turnover associated with energy intake. In addition, neither serum concentration of glucose, FFA, insulin, nor tyrosine/other NAA appears to be the peripheral signal that initiates the sympathetic response. Specifically, when energy intake of a high carbohydrate diet is restricted to 50-64% of ad libitum intake, NE turnover in the heart alone is reduced when mice are fed the restricted intake as a single daily meal presented at the beginning of the dark period. Conversely, when an identical restricted intake is fed as two daily meals presented at the beginning and the middle of the dark period, NE turnover is unaffected in all organs studied. Secondly, fractional NE turnover is not significantly related to any of the serum factors measured.

These results are important for several reasons. First, they separate the effects of food deprivation from those of caloric restriction on NE turnover. Further, the observation of predominance of deprivation over restriction of caloric intake, per se, in eliciting a decrease in NE turnover provides a previously undetermined model for testing possible mechanisms of sympathetic suppression. Insulin (14), and alternatively, tyrosine availability (10,17), have been suggested as the peripheral signals that initiate diet induced changes in sympathetic activity. However, a role for

serum glucose, FFA, insulin or tyrosine/other NAA in mediating the sympathetic response is not supported.

NE turnover in the present study is assessed from the rate of decline of NE concentration after synthesis inhibition by α methyl tyrosine (21). The use of this method relies on the assumption that the rate of decline in NE concentration after synthesis inhibition is equal to the rate of synthesis in untreated animals (30), i.e. that the kinetics of a steady-state system exist. NE concentration declined monoexponentially in all organs studied (brain, heart, pancreas and kidney), except IBAT, demonstrating that one of the major assumptions of steady state kinetics had been met. The decline in NE concentration in IBAT appeared to be biphasic and may underestimate actual NE turnover. These results are similar to those reported in a previous study, where the fit of the data to the monoexponential curve was less significant in IBAT than in other tissues studied, when NE concentration was determined at seven time points over a six hour period (11).

In addition to its limitations in the measurement of IBAT NE turnover, the synthesis inhibition technique may also mask differences in sympathetic activity, given the variability inherent to the technique. Because NE turnover in ad libitum fed mice (Exp. 2-4) was measured at the end of the dark period, in fed animals, and hence is likely reflecting turnover during the thermogenic response to eating, it should therefore reflect maximal diet-induced SNS

activation. This is supported by the observation, in this study, of cardiac NE turnover values similar to those observed in similar mice fed a low protein diet (11), a situation of sympathetic stimulation. In restricted mice, however, NE turnover was measured when the thermogenic response to eating can be predicted to be diminished, since these animals were in a more advanced post absorptive state than were the ad libitum fed mice. The inability of restriction, per se, to affect NE turnover may therefore reflect, in part, the limitations associated with the synthesis inhibition technique, despite its superiority over measurements of NE concentration in blood as an index of SNS activity (31).

Controlling food intake of either the high carbohydrate or the high fat diet to 52kJ (Exp. 1) failed to produce weight gain in mice fed either diet. This level of intake was based on the higher ad libitum caloric intake observed in the preliminary experiment, that of the high fat fed mice, and was intended to prevent restriction in mice subsequently fed this diet in Exp. 1. Experimental animals allowed ad libitum access to high fat diets have been shown to increase caloric intake leading to eventual increases in weight gain and fat storage, unlike high carbohydrate diets which elicit these effects only in older animals (32). Although an early increase in energy intake was observed on day 1 in mice fed the high fat diet, intake over the 3 day period was only 8% above that of control mice fed the high

carbohydrate diet (Preliminary Exp.). It appears that, as soon as food intake is controlled by feeding a fixed amount, some degree of restriction develops, because of spillage or conversion to a meal feeding pattern, i.e. rapid consumption of the limited amount of food. This may account for the failure of protein supplementation to suppress NE turnover in rats compared to chow fed controls (8). Alternatively, seasonal variations in food intake could account for the unintended production of deprivation in these animals.

Energy restriction in Exp. 1 resulted in total body and organ-specific weight loss. Diet composition affected only kidney weight and IBAT endogenous NE concentration. NE concentration was lower in the IBAT of mice fed the high fat diet regardless of level of energy intake. This lower endogenous NE content has been previously reported in the heart of mice fed a high fat diet and tends to support the theory that high fat feeding may alter NE storage (6). Although a lower NE concentration is frequently seen in situations of increased NE turnover (3,6), this does not appear to be the case in the present study (Exp. 1), since the calculated turnover rate (K), which incorporates the lower NE content, was not consistently higher in the mice fed the high fat diet than in those fed the high carbohydrate diet.

The inability of either high carbohydrate or high fat diets to affect NE turnover in the present study (Exp. 1) does not contradict previous observations of fat and

carbohydrate stimulation of sympathetic activity (6-9) as dissimilar experimental conditions exist. Animals in the previous studies were either fed ad libitum (9) or their caloric intake was increased 50-100% over ad libitum intake by isocaloric supplementation of varying amounts of chow intake with fat or carbohydrate (6-8). In the present study, the inability of the relative caloric restrictions of both a high carbohydrate and a high fat diet to affect NE turnover suggests that deprivation itself is a major factor influencing sympathetic activity, or alternately, that changes in NE turnover are not apparent over this range of deprivation.

Thus, Exp. 2 examined the effect of energy restriction without deprivation on NE turnover. Meal-feeding a restricted intake (34kJ) as 2 daily meals failed to affect NE turnover or NE content in any organ although NE turnover tended to be lower in heart, kidney and pancreas of the restricted "meal-fed" mice. The meal-feeding paradigm was planned to isolate the effects of restriction, per se, by decreasing the duration of food deprivation. When deprivation was subsequently produced by presenting the identical restricted intake (34kJ) as a single daily meal, NE turnover in heart alone was 38% lower than in ad libitum fed mice. These results suggest that the duration of deprivation is the predominant determinant of sympathetic suppression, and not the restriction in energy intake, per se. The importance of deprivation as a stimulus for SNS

suppression was again demonstrated by the similarly lower cardiac NE turnover in mice fed a more restricted intake (24kJ) as a single daily meal compared to control mice fed ad libitum or those fed an identical restricted intake as two daily meals (Exp. 4).

The role of 24-48h fasting in suppressing sympathetic activity has been well established (3-5). However, an effect of modest food deprivation as an independent variable in relation to restriction of energy intake, has not previously been identified. An effect of deprivation in determining SNS suppression has been previously suggested by the observation of reduced cardiac NE turnover in mice restricted to 35% of their ad libitum intake (33), presumably presented as a single meal, but the effect was not attributed at that time to deprivation, but rather to energy restriction. A relationship between energy intake and NE turnover is observed in restricted and ad libitum fed mice (Exp. 2-4), when the confounding effects of relative deprivation (Exp. 1) are removed (Table II-15). However, this correlation appears to be a consequence of changes in body weight because the correlation between energy intake and fractional NE turnover disappears when the effect of body weight is controlled.

The functional significance of reduced NE turnover in response to deprivation in the heart alone, is not readily apparent. Diet-induced changes in sympathetic activity are typically associated with widespread effects in peripheral

NE turnover, and have generally been observed in IBAT as well as in heart (4,6,9). Moreover, proposed associations between diet and changes in energy expenditure mediated by the SNS (diet-induced thermogenesis) observed in experimental animals have been based heavily on the thermogenic function of IBAT (34). The limitations associated with the method of NE assessment in IBAT used in this study have already been discussed. In addition, Levin et al (35) suggest that IBAT NE turnover is, in itself, an unreliable index of thermogenic status as increases in IBAT turnover can occur without alteration in the organ's thermogenic activity, and are not always accompanied by tissue hypertrophy or changes in protein or lipid content. It is possible that the duration of deprivation (and/or reduction in caloric intake) in the present study was insufficient to suppress IBAT NE turnover. Moreover, fasting and sucrose overfeeding in rats have been known to produce, respectively, greater suppressive and stimulatory effects in the heart compared to IBAT (4,6,9,36). The greater sensitivity of NE turnover in heart to the dietary manipulation in this study and others may be reflective of the magnitude of the organ's sympathetic innervation, which exceeds that of all other organs studied.

The lower cardiac NE turnover in deprived mice may have no functional significance for thermogenesis or may simply reflect an adaptive response to prolonged deprivation. Cardiac NE β receptors have functional effects on heart

rate, myocardial contractility and peripheral vasodilation (37). A decrease in cardiac NE turnover may therefore represent a sympathetically mediated reduction in blood flow to IBAT. The rate of blood flow to the IBAT is highly correlated to the thermogenic status of the tissue (38). Thus, although the consequences of decreased cardiac NE turnover on blood flow are beyond the scope of the present study, the diet-induced changes observed may yet have some functional significance for IBAT mediated thermogenesis.

A specific role for diet-induced changes in NE turnover in mediating changes in body weight is suggested by this study. NE turnover was highest in the heaviest mice and lowest in those with the greatest weight loss (mice restricted and receiving one meal daily). In addition, in restricted mice receiving two meals per day, both weight loss and NE turnover rate were intermediate between mice fed ad libitum and the restricted mice fed a single daily meal. However, these are associational observations, and the research was not planned to assess the relationship between NE turnover and body weight and fat content.

It has been suggested that sympathetic suppression accompanying fasting could reduce the efficacy of weight reduction with modified fasts in the obese. Although it appears that weight loss was indeed higher in those mice with lower NE turnover, attributing these changes to short-term alterations in cardiac NE turnover would be highly speculative. Moreover, the relevance of these observations

to long-term weight control is not clear and cautious extrapolation to the human condition is warranted. Nevertheless, the inability of restriction with minimal deprivation ("meal-fed" restricted) to affect cardiac NE turnover in the experimental model may have some therapeutic relevance. Cardiac complications have been observed in human subjects refed after a modified fast (39).Landsberg and Young (18,40) suggest that cardiac arrhythmias may be a consequence of sympathetic stimulation during refeeding preceded by sympathetic suppression during fasting. The results of this study suggest that increasing meal frequency during caloric restriction may be beneficial in preventing sympathetically mediated changes in cardiovascular tone.

The results of this study support the proposal by Young and Landsberg that an increase in central NE turnover with fasting stimulates descending inhibitory pathways resulting in peripheral inhibition (14). Specifically, brain NE turnover tended to be higher in all restricted mice than in ad libitum fed mice, with the trend being greatest in the deprived restricted mice. Furthermore, a 30% (NS) higher brain NE turnover was accompanied by a significant suppression of cardiac NE turnover in restricted mice fed one meal only, compared to ad libitum fed controls. In comparison, when an identical restricted intake was fed as two daily meals, brain NE turnover was higher than in controls by less than 1% and cardiac NE turnover was unaffected.

The peripheral signal that coordinates dietary intake and central sympathetic function is not evident from the results of this study. Dietary factors known to stimulate or suppress NE turnover should be accompanied by simultaneous changes in turnover and in the peripheral signal. Insulin, through its effect on glucose metabolism (14), and tyrosine availability (10) have been suggested as alternatives for the peripheral signal. However, the lower NE turnover produced by deprivation or high protein feeding was unrelated to changes in serum concentrations of glucose, FFA or insulin or in tyrosine/other NAA. The inability of blood glucose levels per se to determine the sympathetic response has been previously suggested by the observation of sympathetic suppression in spite of hyperglycemia produced by 2-deoxyglucose, a glucose analogue that impairs intracellular glucose metabolism (32). In addition, fructose produces greater increments in energy expenditure than glucose, despite significantly lower plasma insulin and glucose and similar FFA levels (41). These observations suggest that intracellular glucose metabolism, independent of serum insulin, glucose or FFA concentration is more likely to be the signal that links changes in diet to those in sympathetic activity. Nevertheless, a decrease in serum insulin concentration has been suggested as being sufficient to produce sympathetic suppression (14), but in the present study insulin concentration was not affected by dietary manipulation, although cardiac NE turnover was.

In the present study, serum glucose concentration was more related to caloric restriction than to diet composition or duration of deprivation. Restriction of either the high carbohydrate diet or the high fat diet similarly lowered serum glucose regardless of the duration of deprivation. Although serum glucose was consistently elevated with ad libitum consumption of a high carbohydrate or a high fat diet (known stimuli for sympathetic activation), high protein feeding, which is associated with sympathetic suppression, resulted in only a slightly lower serum glucose level than in the other ad libitum fed mice. The concentration of FFA in serum was likewise more dependent on caloric restriction than on diet composition or duration of deprivation, and was consistently higher when restricted intakes were fed than with all other dietary treatments.

Because duration of deprivation has been shown in this study to be the major cause of low NE turnover with dietary energy manipulation, it was appropriate to relate these changes in serum substrate concentration to the metabolic consequences of deprivation. The post-absorptive period is characterized initially by the stimulation of glycogenolysis and the gradual reduction of hepatic glucose and insulin concentrations (42). This period is dependent on diet composition and generally begins several hours (3-4 with a high carbohydrate meal) after meal consumption (42) and is terminated by either another meal or early starvation. Early starvation is characterized by hepatic glucose release and

free fatty acid mobilization from adipose tissue, changes that are controlled by decreases in glucose and insulin concentration. An increase in FFA concentration results in increased fatty acid oxidation by extrahepatic tissues which decreases glucose utilization and oxidation (43) and spares protein as a source for gluconeogenesis (44). Although these two stages of deprivation represent very different metabolic profiles, the transition from one to the other is gradual. Animals in the high carbohydrate "meal-fed" restricted and high carbohydrate and high fat restricted (deprived) groups may therefore represent points on this "continuum" of deprivation. Moreover, serum concentrations of glucose and FFA are evidently insensitive in differentiating any differences in relative deprivation and effects of diet composition among these groups of animals.

Metabolic homeostasis is maintained by the complex interaction of hormonal effects with metabolic substrates. The inability of alterations in diet composition, energy restriction or duration of deprivation to affect serum insulin concentration is a reflection of this complexity. An elevation in serum glucose is a known stimulus for insulin release; however, an increase in serum FFA is also associated with an increase in serum insulin (45). Insulin concentration is also related to the concentration of amino acids in serum. Serum concentrations of the branched-chain amino acids, valine, leucine and isoleucine, as well as those of phenylalanine and tyrosine, are depressed by

insulin, which increases their uptake by muscle (46,47). However, in the absence of changes in serum insulin, attributing reductions in NAA concentration to this mechanism is speculatory. Moreover, plasma insulin concentration is a function not only of pancreatic release rate, but also of hepatic uptake, factors associated with receptor binding and of extra-hepatic degradation (48). Thus, diet composition, energy intake and deprivation may affect different aspects of insulin metabolism without effect on serum concentration of the hormone.

An increase in brain tyrosine availability subsequent to an increase in availability of the NE precursor in plasma has been proposed to increase central NE turnover with consequent peripheral suppression (17). The association of an increase in the ratio of tyrosine to other neutral amino acid in serum with an increase in brain tyrosine (10) has provided support for this mechanism. However, high protein feeding failed to alter serum tyrosine concentration compared to high carbohydrate or high fat feeding (Exp. 5). Moreover, serum tyrosine concentrations were similarly lower in all restricted mice regardless of diet composition or duration of deprivation than in ad libitum fed mice (Exp. 5).

Tyrosine/other NAA ratio was markedly lower in high protein-fed mice compared to those fed any of the other dietary treatments, due to a two-fold increase in the concentration of other NAA (Exp. 5). This finding does not

support a role for an increase in plasma tyrosine in mediating the low sympathetic activity observed with feeding a high protein diet. A low caloric intake of either the high fat or the high carbohydrate diet provided as a single daily meal did not affect tyrosine/other NAA ratio compared to ad libitum fed counterparts. However, the ratio was lower in the high carbohydrate "meal-fed" restricted group than in ad libitum fed controls. Because protein concentration in the high carbohydrate and high fat diets are identical, these results suggest a relationship between diet composition, other than solely protein concentration, and the tyrosine/other NAA ratio in serum. The findings that changes in serum tyrosine and tyrosine/other NAA ratio are unrelated to changes in NE turnover are supported by the work of others. When diets containing 20% or 40% protein (by weight) are fed, plasma and brain tyrosine concentrations are unaffected and the tyrosine/other NAA ratio is markedly lower in the 40% protein fed mice in spite of peripheral sympathetic suppression in these mice (11).

In conclusion, this study demonstrates that duration of deprivation, rather than energy intake, per se, is the major factor in lowering cardiac NE turnover in response to a caloric restriction to 50-64% of ad libitum intake. The low cardiac NE turnover observed with deprivation in this study and with high protein diets elsewhere (11), is not associated with changes in serum insulin, glucose, FFA or tyrosine or the tyrosine/other NAA ratio. These results may

have implications for the dietary management of obesity such that an increase in the frequency of meals during weight reduction may minimize the fall in SNS activity observed with restriction of food intake, and subsequently reduce the incidence of cardiac complications observed with refeeding and postulated to be associated with an increase in cardiac noradrenergic function.

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III. APPENDICES

Appendix 1

Assay of Norepinephrine

1. Previously weighed frozen tissues were homogenized in 1 mL 0.4 M perchloric acid($HCLO_4$), 20 uL 0.2 M ethylene diamine tetra acetic acid(EDTA), 10 uL 1.0 M sodium bisulfite($NaHSO_4$), and 100 uL $7x10^{-6}$ M 3,4-dihydroxy-benzylamine(DHBA) as an internal standard.

2. Homogenates were centrifuged at 9000rpm for 10 min. (-5-10^{o}C), and aliquots of the supernatants were transferred to 5mL vials containing 35 mg acid-washed alumina and 2mL Tris buffer (pH 8.6).

3. The samples were agitated with a mechanical shaker for 15 min. and the supernatants were removed by aspiration.

4. The alumina was washed twice with HPLC grade water, and the NE and DHBA were then eluted from the alumina with 200 uL 0.2 M $HCLO_4$. Ten uL of the eluate was injected onto the high performance liquid chromatography system.

Appendix 2

Assay of Glucose

10 uL aliquots of serum were injected into a Beckman glucose analyzer.

Appendix 3

Assay of Serum Total Free Patty Acids

1. 0.3 U acyl coenzyme A synthetase, 1.5 U ascorbate oxidase, 0.7 mg coenzyme A, 3 mg adenosine triphosphate and 0.3 mg 4-aminoantipyrine were dissolved in 1 mL 0.05 M phosphate buffer (pH 9), 3 mM magnesium chloride with surfactant and stabilizers. This solution was added to 50 uL aliquots of serum and an oleic acid standard, mixed and placed in a 37°C incubator bath for 10 min.

2. 13.2 U acyl coenzyme A oxidase, 15 U peroxidase and 0.4 mg 3-methyl-N-ethyl-N-β-hydroxyethyl-aniline dissolved in 2 mL 0.3% phenoxy ethanol with surfactant were then added to the samples, which were subsequently mixed and again placed in a 37°C incubator bath for 10 min.

3. After equilibration with room temperature for 5 min., the optical densities (absorbances) of all samples were read at 55 nm on a Perkin Elmer Spectrophotometer (Lambda 38 UV/VIS,) against a reagent blank.

4. The concentrations of FFA were determined from the following equation:

$$\frac{A_{sample} \times C_{standard}}{A_{standard}}(mmol/L) = C_{sample}(mmol/L)$$

A = Optical density at 550 nm
C = FFA concentration (mmol/L)

Appendix 4

Analysis of Serum Large Neutral Amino Acids

Preparation of serum samples

To 20 uL serum. 200 uL ethanolamine (the internal standard) and 200 uL were added. Samples were vortexed and 500 uL 5% tricarboxylic acid were added. Samples were centrifuged at 3000 rpm for 10 min. and 300 uL saturated potassium borate and 200 uL water were added to the supernatant.

Chromatography System

Samples were mixed using a modified Technicon autosampler and a Chemlab peristaltic pump with a stainless steel mixing tee. Samples were mixed 1:1 with the formaldehyde reagent prior to injection and delay time was 12 sec. Samples were injected using a Valco autoinjector valve equipped with a 20 uL loop. The column used was a Supelcosil 3 uM LC-18 reverse column and a guard column packed with Supelco LC-18 reverse phase packing. Chromatographic peaks were recorded using a Fisher recorder, and integration of the peaks accomplished using a Hewlett Packard 3353 data system with a Hewlett Packard 18652A A/D converter. Analysis time per sample was 49 min.

Preparation of Fluorescence Reagent

One g of o-phthaldialdehyde (OPA) was dissolved in 25 mL methanol; 224 mL 0104 M sodium borate buffer (pH 9.5) were added along with 1.0 mL 2-mercaptoethanol and 20 mL Brij 35.

Gradient Conditions

Solvent A was prepared by adding 11.5 glacial acetic acid and 8.0 g sodium hydroxide to 1600 deionized water. The pH was adjusted to 7.2 with 5 M sodium hydroxide. 180 mL methanol and 10 mL tetrahydrofuran were added and the volume adjusted to 2 L with deionized water. Solvent B was methanol. Flow rate was 1.1 mL/min.

Appendix 5

Analysis of Serum Insulin

1. To 100 uL aliquots of serum and standards, 50 uL $Insulin^{125}$ and 50 uL antibody (rat antiserum) were added. Samples were agitated then incubated overnight at room temperature.

2. Samples were again incubated at room temperature for half hour after addition of 2mL decanting suspension (anti-rat IgG) and centrifuged at 1500 g for 10 min. The radioactivity of the supernatants was determined as follows:

   A. The counts (B) for standards and unknowns were expressed as a percentage of the mean counts of the "0-standard" (B_o):

   $$\% \text{ activity bound} = \frac{B_{(\text{standard or unknowns})}}{B_o} \times 100$$

   B. Sample concentrations of insulin were determined from a standard curve of the % activity bound plotted against the standard concentrations.

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PUBLICATIONS

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Johnston, J.L. and Davis-Street, J.E. Effect of energy intake on central and peripheral norepinephrine turnover in lean mice. FASEB, Las Vegas, Nevada May, 1988 (Abstract)

PRESENTATIONS

Davis, J.E. and Johnston, J.L. Effect of energy restriction on central and peripheral norepinephrine turnover in lean mice. Canadian Federation of Biological Sciences, Winnepeg, Manitoba June, 1987.